CEREAL CHEMISTRY

Published bi-monthly by

The American Association of Cereal Chemists

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Entered as second class matter at the post office at Minneapolis, Minn., under the Act of August 24, 1912. Acceptance for mailing at special rate of postage provided for in paragraph (d-2), Section 34.40, P. L. & R. of 1948, authorized February 16, 1924. Published at 500 So. 5th St., Minneapolis, Minn.

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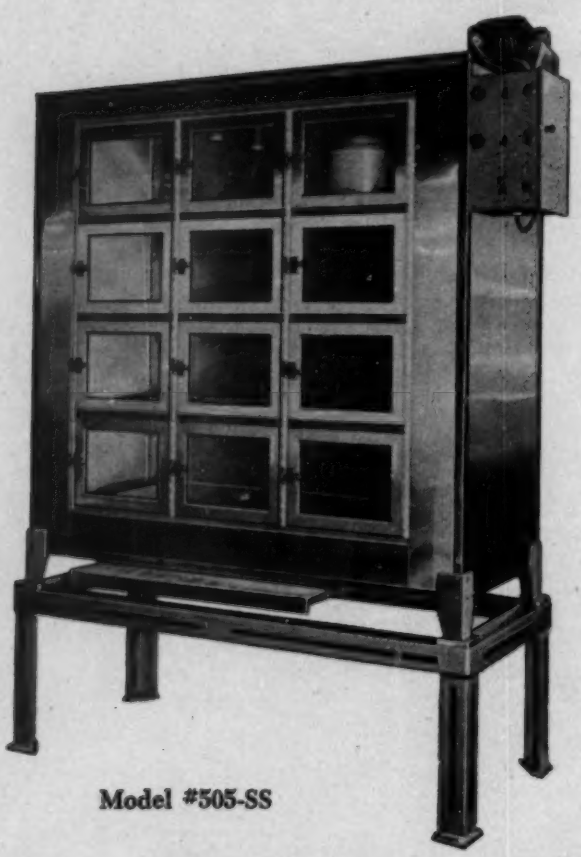

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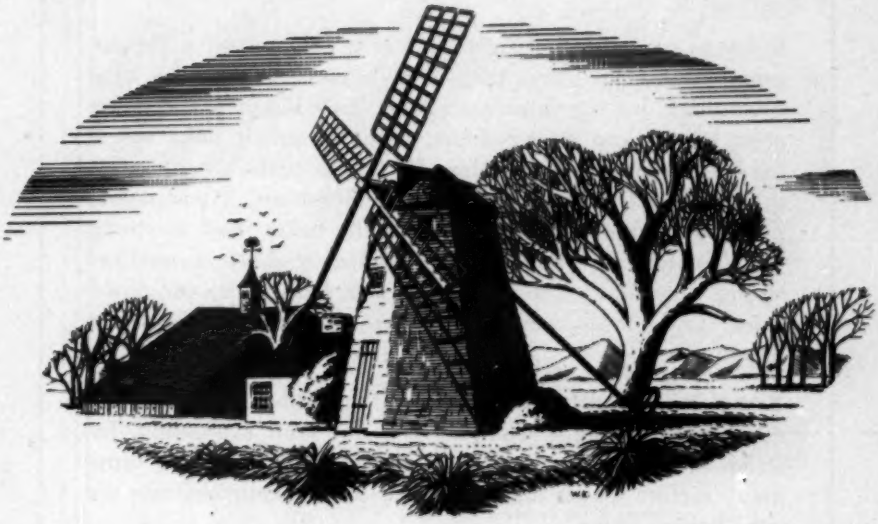

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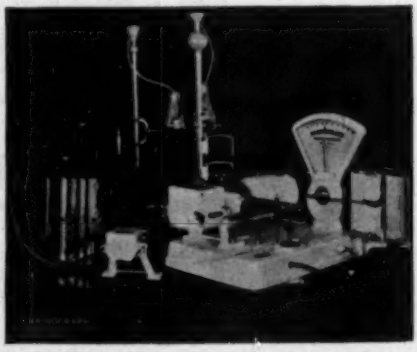

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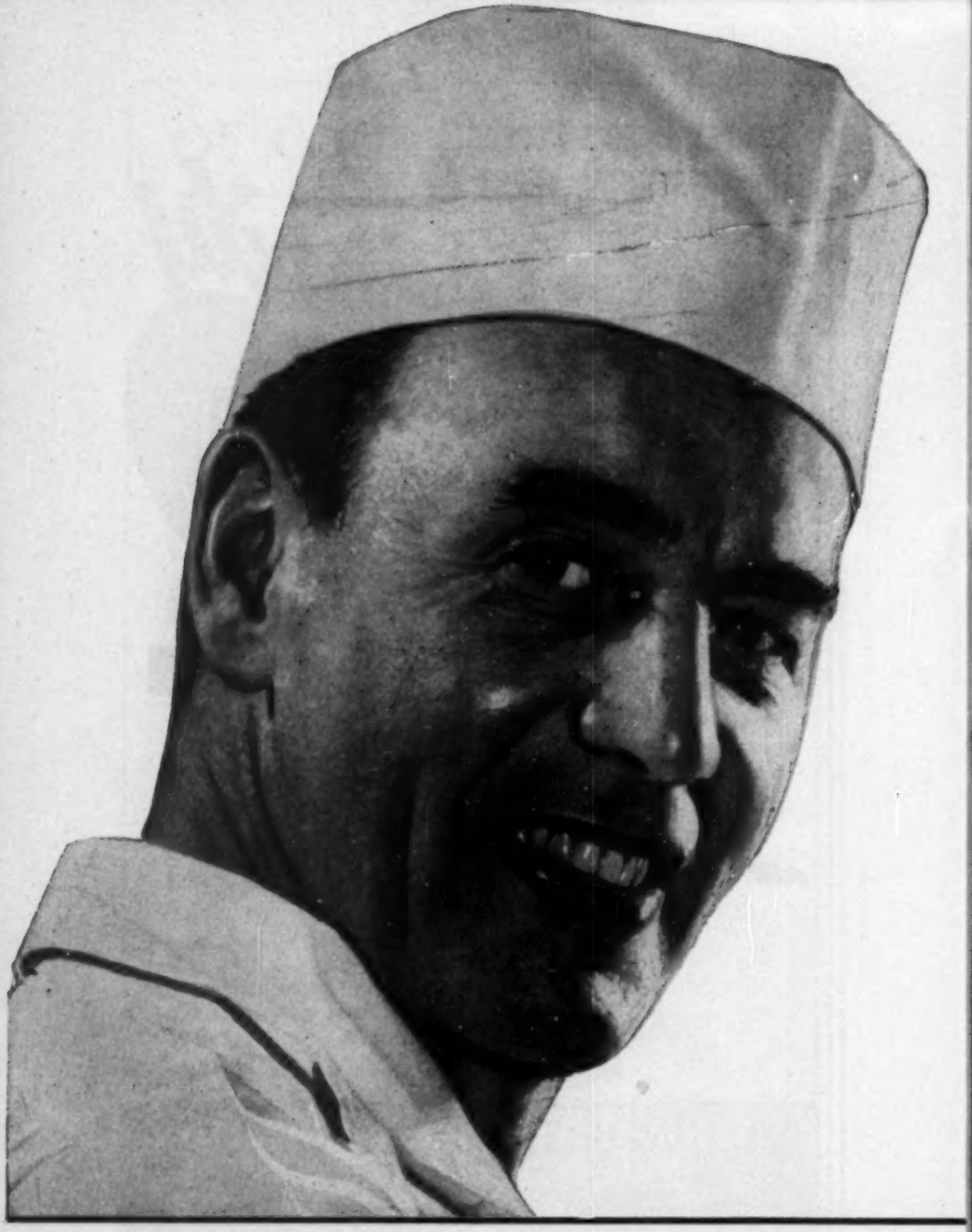

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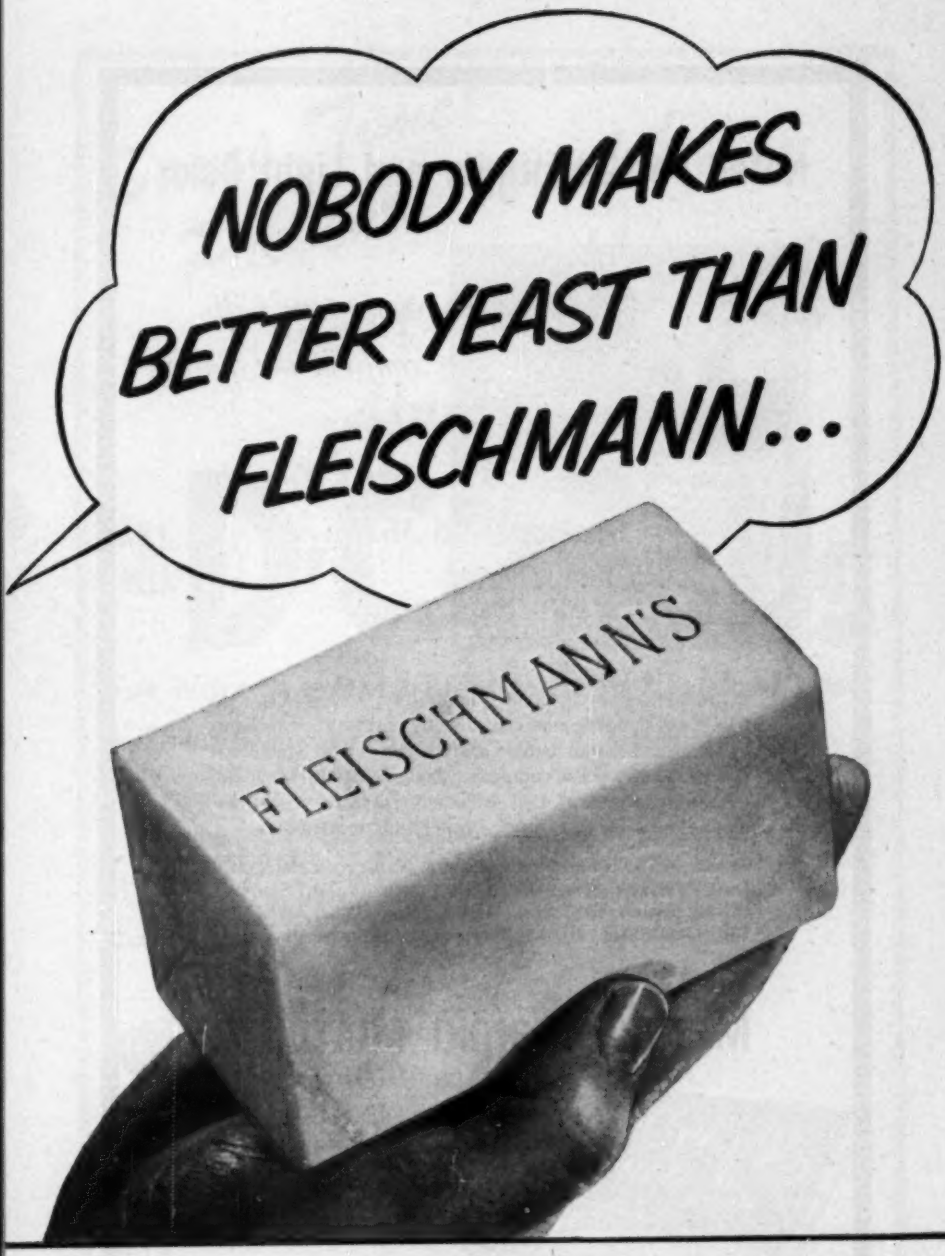

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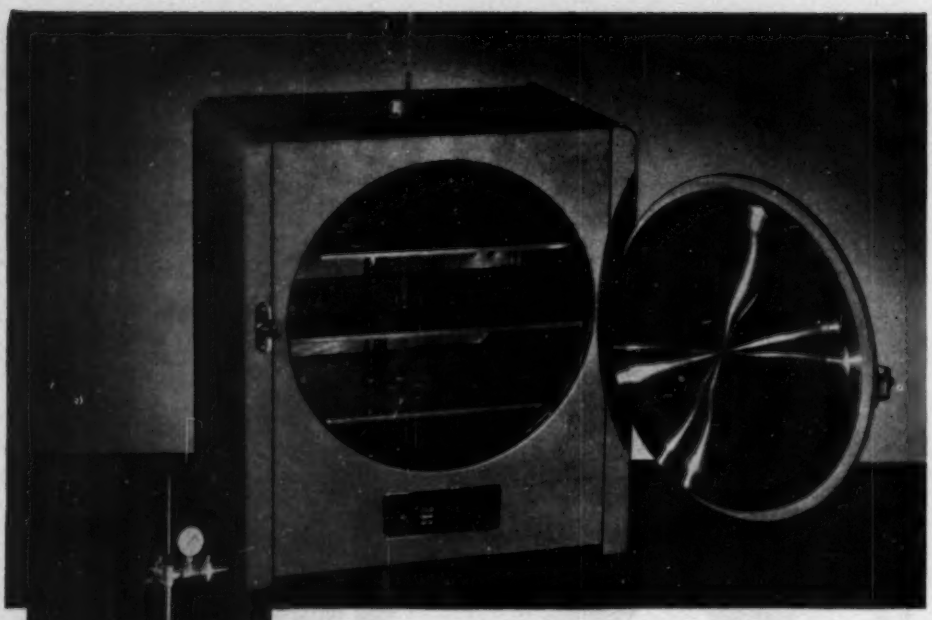

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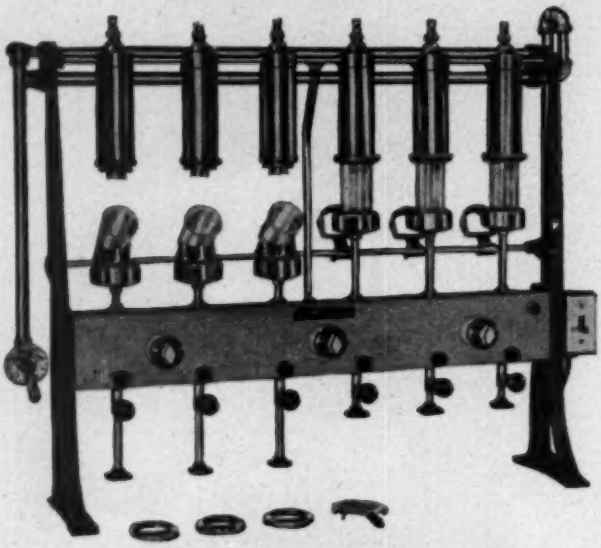

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CEREAL CHEMISTRY

VOL. XXX SEPTEMBER, 1953 NO. 5

SOME VISCOELASTIC PROPERTIES OF WHEAT GLUTEN[1]

DOYLE C. UDY[2]

ABSTRACT

Some of the rheological properties of "purified" wheat gluten have been investigated with the aid of a Jolly balance which has been extensively modified to accommodate relaxation and extension measurements. Gluten from several varieties of wheat has been subjected to numerous treatments—resting for different periods at various temperatures, mechanical working with and without subsequent resting, and conditioning in different salt solutions. These conditioning treatments were made prior to relaxation and stress-strain measurements.

Preliminary results suggested use of two values for indexes of comparison. A linear relationship was found between stretching force and relative length for these gluten samples. The slope of this function is used as the major index for characterizing the various glutens. Also the time required for an imposed stress on a given sample to decay to 1/e of its initial value is used as an index and is called the "apparent" relaxation time after the definition of this constant for materials exhibiting Maxwellian behavior, i.e., viscous flow superimposed on an elastic element.

The glutens become more resistant to stretching after resting, as contrasted with doughs which mellow and soften as a result of relaxation of their internal stresses during resting. It is suggested that new associations between the protein molecules account for the increase in strength during resting or mechanical working of "purified" gluten.

Variations in elastic and viscous behavior for different wheat-flour doughs has long been recognized as a quality characteristic—particularly in the baking industry. The underlying reasons for these viscoelastic variations are not fully understood. Consequently, the methods which have been developed for evaluating and characterizing these properties in doughs or dough derivatives are quite numerous and somewhat varied. A convenient and accurate method of quantitatively measuring these physical properties would be of value where quality is an important factor. Most of the machines heretofore devised for

[1] Manuscript received October 28, 1952.
Contribution of the Western Wheat Quality Laboratory, Department of Agricultural Chemistry, Washington Agricultural Experiment Stations; and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agriculture Research Administration, U.S. Department of Agriculture.
[2] Assistant Chemist, Western Wheat Quality Laboratory, Division of Chemistry, Agricultural Experiment Station, Pullman, Washington.

these purposes have been adequately described and reviewed in the literature (3, 6, 9). Hlynka and Anderson (8) have recently explored the relaxation of external stress on dough and its major constituents with a newly constructed apparatus called a relaxometer. Another apparatus and a procedure for measuring some viscoelastic properties of "purified" wheat gluten will be discussed in this paper.

Measurements of viscoelastic response for several gluten preparations indicate that the amount of mechanical work done on the gluten, the time of resting before measurement, and the temperature of the buffer solution in which the gluten is rested have pronounced effects on the physical character of the gluten. The slope of the stretching force-relative length curve and the relaxation time have been used as indexes of comparison for the various glutens under a number of different conditions. It is hoped that these observations may help in the understanding of the mechanisms operating in doughs or glutens and will throw some light on the interpretations previously reported by use of other gluten-testing machines.

Materials and Methods

Description of Gluten-Testing Apparatus. Figure 1 shows a schematic diagram of a modified Jolly balance. This balance, normally used for specific-gravity determinations, can conveniently be used to measure the force required to elongate a gluten sample to any desired relative length. Also, it is suitable for measuring the decay of the stretching force while the sample is held in a fixed, elongated position, and enables measurement of a period of relaxation.

The gluten sample *1* is floated in a buffer solution with the help of corks *2*. Since the "purified" gluten has a density just slightly greater than the buffer solution, the upper cork is adjusted with weights so that it will just float. The lower cork is fastened to a glass hook in the bottom of the glass tube *3*. The glass tube is supported by rubber bands attached to a cross arm which is in turn fastened to the movable index marker *4*. A stationary vernier *5* is located at the top of the main supporting column *6*. A movable, inner column *7* operates with a rack and pinion and is powered by a constant-speed, reversible electric motor *8*. Another vernier *9* is located atop this movable column. A second movable inner column *10* is located within the first movable column and is held in any position relative to column 7 by a thumbscrew *11*. An extension rod *12* is fastened to the sliding scale *13* and also to the top of the second inner column. The cord *14* is tied to the inside of the lower end of the traveling column *7* and is

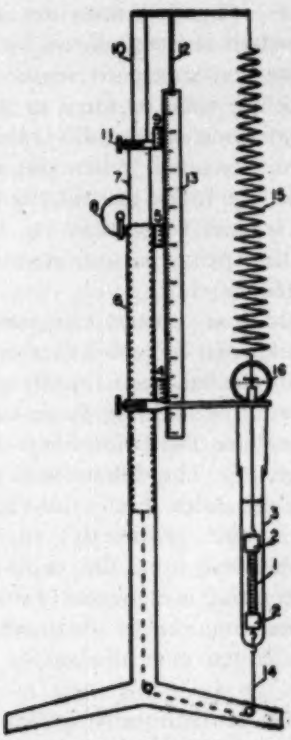

Fig. 1. Schematic diagram of a modified Jolly balance for measuring some mechanical properties of gluten.

guided, by two small pulleys at the base, up to the bottom of the glass tube.

The cord can be tied directly to the hook at the bottom of the glass tube, causing the tube to move at the same speed as the inner column, or it can be looped over the hook and tied at the base support which will move the tube at one-half the speed of the inner column. Tension on the rubber bands permits the glass tube to move up or down at a constant rate with the motor.

The upper cork is fastened by a detachable hooked wire to another wire provided with a disc as an index marker. The coiled spring *15* is attached to this wire carrying the index at one end and to an arm located at the top of the movable column *10*. A copper tube fitted on the movable index marker, just in front of the mirror *16*, acts as a stop for the index disc. The upper end of the gluten sample can thus be held in a stationary position while the lower end is being moved downward with the constant-speed motor.

Operating Procedure. The determination of the length of the gluten sample at any instant is accomplished by starting a stop watch simultaneously with the constant-speed motor at the beginning of the measurement. Since the time required to move the glass tube a given distance is known, one can readily calculate the extent the gluten sample has been elongated. When the tension on the spring is adjusted to barely lift the index disc off the copper tube stop, the reading on the sliding scale is proportional to the force required for any elongation, since the spring is calibrated and used only in the region where it obeys Hooke's law.

The relaxation of stress at constant elongation is easily measured by use of the sliding index marker which carries a pointer for reading the scale. After the sample has been rapidly elongated to a predetermined point, the required holding force can be determined by adjusting the sliding scale to the point where the index disc is just clear of the copper tube stop. The difference in this scale reading and the scale reading when the index disc is just clear of the stop, before any elongation of the sample, is directly proportional to the force. The force value is obtained from the calibration curve of force against the distance the spring is elongated. From these measurements an "apparent" relaxation time can be obtained by assuming that the mechanism within the gluten is equivalent to a Maxwell element—to be described later.

Preparation of Samples. Uniformly milled and blended wheat flour (about 100 g.) is formed into a rather stiff dough in a small dough mixer by adding a limited quantity of buffer solution[3] and mixing for a short time. A relatively large quantity of buffer solution is then added, and after a brief mixing the starchy liquid is decanted off. After several repetitions a crude gluten relatively free of starch is obtained. The gluten and starch of hard and soft varieties can be readily separated by this procedure within 5 to 10 minutes.

The crude gluten is "purified" by a modified Lusena (10) procedure. Approximately 40 g. of wet gluten are dispersed in 200 ml. of 0.03 *N* acetic acid[4] with a Waring Blendor. Five minutes are sufficient to completely disperse the gluten. The omission of n-octyl alcohol as an antifoam agent at this point is important, since even one or two drops cause extensive modification of the gluten, as evidenced

[3] The buffer is a 0.05% aqueous solution of an equal weight percent mixture of Na_2HPO_4 and NaH_2PO_4, having a pH around 7.

[4] Experiments carried out by Mr. Clarence R. Bresson of this laboratory show large variations in the dispersibility of glutens obtained from different varieties of wheat. The same dispersibility pattern is obtained at several acid concentrations, using acetic and hydrochloric acid dispersions. In connection with these measurements, the acid binding capacity (9) of gluten from several varieties of wheat was determined and found to be essentially the same and in close agreement with values reported in the literature.

by the load-relative length measurements to be described. The dispersion is next centrifuged in an International Centrifuge for 10 minutes at 3000 r.p.m., after which it is decanted from the starch residue and neutralized to a pH of 6.5 with 0.1 *N* sodium hydroxide. The regenerated gluten is washed with the buffer solution, using a minimum of manipulation.

Small, roughly cylindrical shapes of "purified" gluten are molded by hand and freed of air bubbles. These cylindrical pieces are allowed to relax in the buffer solution at 25° C. for 5-10 minutes; they are then placed on a small glass plate and frozen at —20° C. Variations of this method were to rest the gluten cylinders longer and at different temperatures in the buffer solution before freezing, and also to work the gluten with a spatula or with the dough mixer for a short time before resting and freezing.

The frozen gluten cylinders, when allowed to thaw slightly at room temperature, become semi-hard and can be easily cut into a uniform rectangular shape, 4 mm. to the side, with a cutting tool designed for this purpose. The rectangular block of gluten is cut to a length of 2.5 cm. and then placed in the buffer solution for about 1 minute. The gluten is then secured to the corks, placed in the buffer solution in the glass tube, and a stretching measurement made. A good bonding between the gluten and cork is achieved by application of a thin surface of ground dried gluten on the end of the cork before contact is made with the gluten sample.

Moisture and Fat Determination. The moisture content of the rested gluten samples was determined by the method of Hlynka and Anderson (9). The surface moisture of the sample was removed by hand manipulation. After the sample was weighed in a crucible, enough 95% ethyl alcohol was added to cover the sample. After a few minutes, the crucible was placed in an oven at 130° C. Drying was complete in about 3 hours.

The fat content of the dried gluten samples was determined as follows: The sample was extracted for 15 hours with ethyl alcohol in a Soxhlet extraction apparatus. The extract was then dried carefully in an oven, reextracted with chloroform, filtered, and finally dried at 60° C. in the oven.

Results and Discussion

Stress-Strain Relationship. The work of Schofield and Scott Blair (12) indicated that the "purified" gluten might closely simulate a Maxwell element (1), i.e., the viscous flow would be superimposed

upon the elasticity. The total rate of change of deformation could then be given simply by the summation:

$$d\gamma/dt = 1/\eta\,(S) + 1/G\,(dS/dt) \qquad (1)$$

where $d\gamma/dt$ denotes the rate of strain, η the viscosity, S the shear stress and G the shear modulus. Under these conditions, the relaxation time of a gluten sample could be determined by measuring the decay in stress at constant deformation, i.e., when $d\gamma/dt = 0$. The solution of the resulting differential equation is:

$$S = S_0 \exp.\ (-G/\eta)\ t = S_0 \exp. - t/\tau \qquad (2)$$

time, which is equivalent to η/G, and is defined as the time required for the initial stress to decay to the value S_0/e.

If we again consider the gluten as a material possessing both instaneous elasticity and a superimposed flow and subject the system to a constant rate of deformation, then the solution to equation (1) gives us the stress-strain relation:

$$S = R\eta[1 - \exp. - (G/R\eta)\gamma] \qquad (3)$$

where R represents the particular rate of strain, $d\gamma/dt$, imposed.

The initial slope of the stress-strain curve (i.e., where gluten essentially exhibits only elastic behavior) is Young's modulus for that particular rate of extension. However, if the gluten flows appreciably during the extension a part of the work is dissipated by this plastic mechanism, and the slope of the stress-strain curve (including its initial portion) is no longer related to Young's modulus in a simple manner.

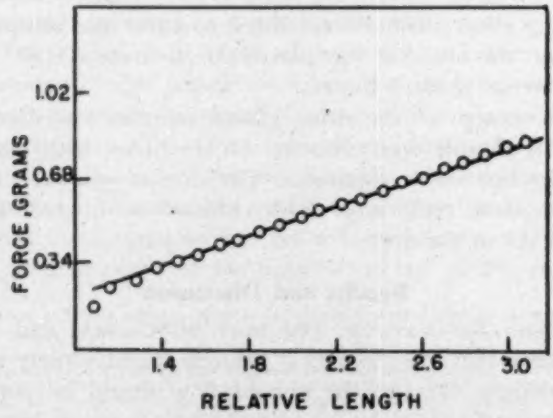

Fig. 2. Relationship between the stretching force and the extent of stretching for "purified" gluten.

Although the measurement of the initial stress values at constant rates of strain was not considered precise enough to calculate the elastic modulus of individual gluten samples, the data obtained did show some interesting and possibly useful relationships.

The stretching force was found to be directly proportional to the relative length of the sample (see Fig. 2) in the range of 50 to 250% elongation. As mentioned above, the measurements are not reliable in the range 0 to 50% elongation. The slope of the plotted straight line is a function of the rate of elongation, as would be expected of a viscoelastic material (2). Therefore, it is pointed out that all slope values referred to in this discussion were measured at a constant deformation rate of 0.0191 cm./sec.

Figure 3 shows a typical stress-relative length curve for "purified"

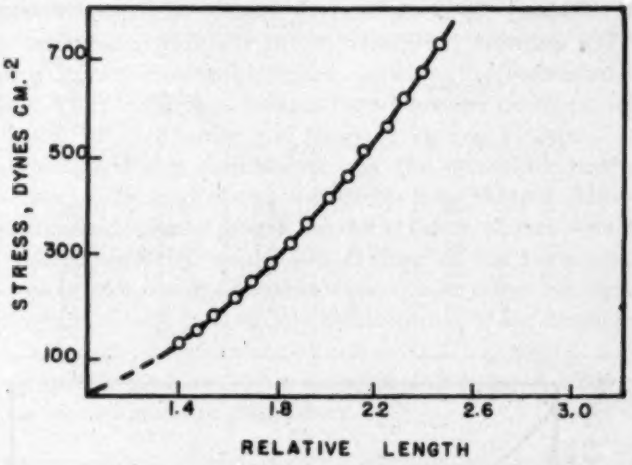

Fig. 3. The stress-relative length plot of a typical "purified" gluten sample.

gluten. The stress at a given elongation is the quotient of the measured stretching force divided by the area of cross-section of the sample, assuming no decrease in volume of the gluten. The density, from which the volume was calculated, was determined indirectly for each sample by measuring its specific gravity on the Jolly balance. It is of interest to point out that the theoretical stress-strain curve, equation 3, differs from the experimentally determined stress-relative length curve. Equation 3 yields a curve that is concave downward and reaches an asymptotic value of R_η, while the curve in Fig. 3 is concave upward. This divergence between theory and experimental results is likewise apparent in the relationship between the relaxation time and

the slope of the force-relative length curve, as will be pointed out later in the discussion.

For a given sample of flour, the slope values on "purified" gluten were found to agree quite satisfactorily. The slopes obtained for each gluten under the designated conditions are shown in the results given in Table I. The values are averages of four measurements on each of at least three completely separate gluten preparations from the same sample of flour for a single variety. The precision is ± 0.02 in the slope value.

The data presented in the second and third columns are of general interest in connection with the stretching measurements.

TABLE I

COMPARISON OF THE STRETCHING FORCE–RELATIVE LENGTH SLOPE VALUES OF GLUTEN FROM SEVERAL VARIETIES OF WHEAT TOGETHER WITH OTHER PROPERTIES

Variety	Moisture Content	Fat Content	τ	$dF/d\gamma$		
				Before Resting	After resting 1 hr. at 25°C.	After resting 2 hrs. at 25°C.
	%	%	*min.*	g.	g.	g.
Idaed	67	...	8.0	0.13	0.26	0.27
Rio	67	9.1	14.0	0.29	0.40	0.42
Elgin	68	11.0	6.5	0.12	0.21	0.22
Wasatch	67	...	6.5	0.14	0.24	0.24
Comanche	68	8.2	9.0	0.31	0.39	0.40
Golden	67	11.6	9.5	0.24	0.33	0.34
Chiefkan	67	...	7.0	0.19	0.22	0.15

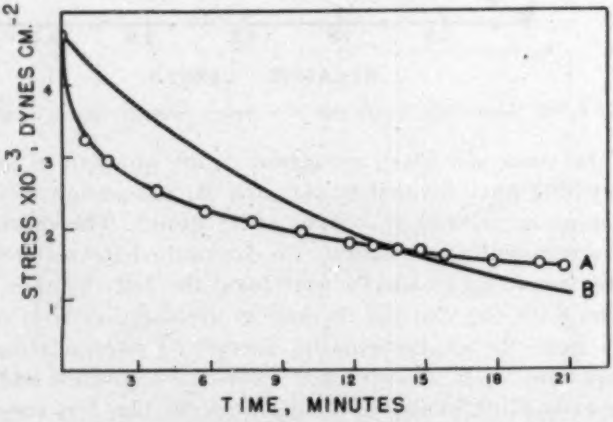

Fig. 4. Decay of stress as a function of time for wheat gluten. A. Curve drawn through experimental points. B. Theoretical curve, $S = S_0 e^{-t/\tau}$ based on the assumption of a Maxwell element for $\tau = 14$ minutes.

Apparent relaxation times were obtained for each of the "purified" glutens by elongating the sample to 150% of its initial length and measuring the initial stress, S_0, imposed. The time for this stress to decay to 1/e of its value was measured and is recorded as the "apparent" relaxation time or τ in Table I. A stress-time plot of the experimental values indicates that decidedly shorter and substantially longer relaxation times exist for a given gluten sample when compared with the theoretical curve of equation (2) which assumes Maxwellian behavior. Accordingly, the experimental curve would indicate a distribution of relaxation times (see Fig. 4).

When the stress is plotted against the logarithm of time for these experimental values, a linear relationship is obtained. This is in accordance with and substantiates the recent work of Hlynka and Anderson (8). A theoretical basis for such behavior is lacking. The relaxation of internal stresses in resting dough conforms to Maxwellian behavior as pointed out by Dempster, Hlynka, and Winkler (5), and a linear relationship exists between the logarithm of stress and time. The "apparent" relaxation time may, however, serve as a useful index for comparing and studying various glutens.

It seemed desirable to find out how the relaxation time and the slope values of the load elongation curves were related. Although the slope of the load-relative length curve is empirical, one would expect it to be directly related to the initial slope of the stress-strain curve or modulus, except for the cross-section correction on the sample. Accordingly, τ should be inversely proportional to the modulus G and hence to the slope, $dF/d\gamma$, since by equation (2), $t = \eta/G$.

The experimental results as depicted below for a single variety, Idaed, do not conform to this theory.

τ (min.)	$dF/d\gamma$ (gr.)	Temp. (°C.)	Resting time (hrs.)
4.0	0.13	..	0
7.0	0.26	25	1
10.0	0.33	35	1

The apparent relaxation time shows an increase with increasing slope values. The temperature and resting time are variables which were adjusted to give a wide range of slope values, and will be elaborated upon later in the discussion. A possible explanation for this behavior may be that the viscosity does not remain constant but increases relatively more than the modulus upon resting of the gluten. The increased viscosity could be the result of electrostatic and other inter-

actions between appropriate sites on the molecules which have come into position during resting.

It should also be kept in mind that $dF/d\gamma$ is an empirical value and not the true modulus. It is undoubtedly also some function of the viscosity and would, therefore, be expected to deviate from the formal theory. It was also pointed out above that the initial slope of a stress-strain curve can only be interpreted as the modulus of elasticity under certain conditions.

Some viscosity measurements of gluten dispersed in acetic acid do indeed show a marked correlation with the $dF/d\gamma$ values on these flour samples[5].

The work of Schofield and Scott Blair (12) also points out that ordinary wheat-flour dough obeys the Maxwell relationship, equation 1, and shows an exponential decay of the stress at a constant elongation. The fact that the "purified" gluten does not follow this relationship would lend supporting evidence to the hypothesis (7) of a carbohydrate-protein interaction which would definitely affect the stress-strain behavior of gluten in a flour-dough system.

In this connection it is also of interest to observe that each "purified" gluten sample has the same capacity for holding water as is evidenced by the moisture-content measurements recorded in Table I. With flour doughs, it is generally recognized that the water absorptions are quite markedly different for flour from different varieties of wheat at equal protein levels.

Effect of Lyophilizing. During the development of a satisfactory method, several interesting properties of gluten were observed. At the outset it seemed desirable to lyophilize the "purified" gluten in order to have a large supply of stable material available for testing. Although a single preparation of the lyophilized gluten was remarkably stable and self-consistent, successive preparations were not consistent in respect to the load-elongation measurements, and they could not be duplicated satisfactorily. This is probably a result of excessive working during freezing and drying and also of the varying amounts of denaturing. A similar situation of inconsistency was observed by this laboratory in viscosity measurements of lyophilized gluten (13). Each individual preparation was consistent only within itself. Lyophilizing was therefore abandoned.

Effect of Washing Solution. When distilled water was used for washing out the gluten, discrepancies were noted for some varieties at the step where the neutralized gluten is washed. Some of the glutens would lose a fair amount of cohesiveness at this point, and any true

[5] Unpublished results of this laboratory.

characteristics seemed to be lost or considerably altered in subsequent measurements. Either tap water or a buffer solution effectively corrected these difficulties—presumably by supplying the necessary ions to maintain the proper solubility conditions. For uniformity of the procedure, the buffer solution was used throughout in the preparation of gluten samples. In order to test the effect of the buffer salts on the slope values, a 2% common salt solution was used to wash out the gluten. Mecham and Weinstein (11) have recently shown that the lipid content of crude gluten is considerably lowered by the incorporation of salt in the washing procedure. The effect on the stretching force-relative length curve for the gluten prepared from Idaed with this saline washing solution was to consistently decrease the slope from 0.26 to 0.19. This indicates that the gluten is weakened by the loss of lipids and is presumably the result of a decrease in the intermolecular interactions between protein molecules and lipo-protein molecular complexes.

Effect of Resting or Working. It was also observed that a change in the gluten was taking place as it rested longer in the buffer solution. A state was reached after about 45 minutes, however, when the rate of change decreased sharply. Usually 2 to 3 hours' additional resting was required to bring about further significant changes, as evidenced by load-elongation measurements. The same stationary state could be approached much more readily by working the gluten, with a spatula or with the dough mixer. Working for 5 or 10 minutes produced the same result as 45 minutes of resting (see Table II). Several measurements on various glutens indicated that this was a general characteristic and not peculiar to any one variety. An hour of resting after being worked 5 minutes did not bring about any further increase in the slope value.

TABLE II

COMPARISON OF MECHANICALLY WORKED GLUTEN WITH RESTED GLUTEN IN RESPECT TO THE SLOPES OF THEIR STRETCHING FORCE–RELATIVE LENGTH CURVES

Variety	$dF/d\gamma$ (gr.)						
	After 1 Hr. of Resting	Worked in Dough Mixer			Worked with Spatula		Before Resting or Working
		1 min.	3 min.	5 min.	5 min.	10 min.	
Idaed	0.26	0.21	0.22	0.24	0.26	0.26	0.13
Wasatch	0.24	0.19	0.22	0.23	0.22	0.24	0.14

Longer periods of resting showed that a maximum strength was attained. This varied from 2 hours to 20 hours, depending upon the

variety. For example, it can be seen in Table I that Chiefkan has reached a maximum strength in less than 2 hours of resting. Elgin, on the other hand, did not reach a maximum slope value even after 20 hours of resting (not evident in the table.) Proteolytic action would probably account for some of the weakening effect.

It should also be mentioned that the rested gluten samples could be stretched from 20 to 30 times their initial length before they ruptured. If a gluten sample is allowed to relax immediately after a 150% elongation on the machine, the amount of viscous flow can be determined. This was usually about 5%. In other words, the gluten sample will return to very nearly the same initial length after this extent of stretching.

Effect of Temperature. Another variable which affected the gluten, and consequently the stretching force-relative length curve, was the temperature of the buffer during resting.

Table III gives a summary of the effect of temperature during resting upon the strength of the gluten. At the lowest temperature, 15°C., the $dF/d\gamma$ values are the same or only slightly different from the corresponding values for these two samples, when measured before any resting period, as given in Table I. Otherwise, a fairly uniform increase in $dF/d\gamma$ is noted as the temperature of the solution in which the glutens are rested is increased.

TABLE III

THE EFFECT OF TEMPERATURE UPON THE LOAD–RELATIVE LENGTH CURVE FOR GLUTEN FROM TWO DIFFERENT SAMPLES OF FLOUR. ALL OF THE SAMPLES WERE RESTED ONE HOUR IN BUFFER SOLUTION AT THE SPECIFIED TEMPERATURE

Temp. (°C.)	$dF/d\gamma$ (gr.)	
	Idaed–49022R	Elgin–51003
15	0.15	0.13
20	0.20	0.17
25	0.26	0.21
30	0.29	0.23
35	0.34	0.26

A possible mechanism for the effect of working and of resting for various periods and the effect of temperature on the gluten samples could be the progressive formation of weak electrostatic associations as the molecules come into the proper position or configuration. At the higher temperature the protein molecules are more mobile. This would account for a higher limiting initial increase in the $dF/d\gamma$ value. The relative time of resting does not appear to be important at the lower temperature, since here the molecules are not active

enough to form many new associations and the gluten sample as a whole is not strengthened appreciably.

The increase in relaxation time with increasing $dF/d\gamma$ values as given earlier is reasonable in this respect, since the viscosity is related to secondary electrostatic associations and other forces of attraction between protein molecules.

The length of time that the gluten remained frozen at −20°C. did not alter the values obtained over a period of 4 days. This, of course, required that the gluten cylinder was big enough to enable one to cut out the rectangular sample free from any dried gluten at the outer edge.

Measurements on a Single Variety. A single pure variety, Elgin, grown at several locations and varying in protein content, gave the $dF/d\gamma$ values as shown in Table IV, for crops grown in three consecutive years.

TABLE IV

VARIABILITY OF THE SLOPE VALUE FOR GLUTEN FROM THE ELGIN VARIETY OF WHEAT GROWN IN DIFFERENT LOCATIONS AND IN DIFFERENT YEARS

Lab. No.[1]	Location	Flour Protein	$dF/d\gamma$ (gr.) (After resting 1 hr. at 25°C.)	τ
		%	*g.*	*min.*
49006V	Pullman	8.9	0.24	6.0
49006M	Moro	7.0	0.59	10.0
50014	Lind	6.8	0.57	10.0
50049	Pullman	7.5	0.52	8.5
50073	Moro	7.8	0.40	6.5
51021	Lind	8.3	0.51	8.5
51003	Pullman	10.3	0.21	5.5

[1] The first two figures designate the year grown.

The results of these measurements would indicate the the "purified" gluten from a given variety is not necessarily identical in its physical properties, but varies with the growing environment and is determined by some other unknown factors. This would help to account for the fact that no correlations between the intervarietal slope values of Table I and other better-known dough properties appear to exist.

Further work is in progress in an effort to interpret more fully the meaning of the slope values of "purified" gluten as measured in these studies.

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EFFECT OF MOISTURE CONTENT ON THE STABILITY OF A YELLOW CAKE MIX COMPOUNDED WITH PLASTIC AND POWDERED SHORTENINGS[1]

C. S. McWilliams and T. Soloski

ABSTRACT

Prepared cake mixes for use by the Armed Forces must be capable of withstanding prolonged storage under the adverse conditions frequently encountered in overseas supply channels. To determine the role of moisture in deterioration of yellow cake mix, a series of cake mixes were compounded with an undehydrated flour (11.7% moisture) and the same flour was dehydrated to three moisture contents (8.0, 2.8, and 2.1% respectively), a plastic shortening and two types of commercially available powdered shortening products being used, respectively. The mix samples were stored in hermetically sealed containers at 100°F. (37.8°C.). Representative portions were test-baked at monthly intervals and free fatty acid content and peroxide values were determined on fat extracted from the samples at bimonthly intervals. Cake mixes compounded with flour of normal moisture content (11.7%) deteriorated in 4 to 6 months, whereas those containing 8.0% moisture flour deteriorated in 7 to 8 months. Mixes compounded with flours containing 2.8% moisture or less performed satisfactorily after one year's storage. Cake mixes compounded with flours having normal (11.7%) and 8.0% moisture and powdered shortening were more stable than mixes compounded with identical flours and plastic shortening. The rate of free fatty acid development in mixes compounded with 8.0% moisture flour was significantly lower than in mixes compounded with flour at normal moisture content (11.7%). Development of free fatty acid in mixes compounded with flours containing 2.8% moisture or less was arrested. Peroxide values showed no correlation with storage deterioration.

Prepared cake mixes are advantageous to military feeding operations because they assure production of uniformly acceptable baked items, simplify logistical problems, and reduce requirements as to baking skill and equipment. In military overseas supply channels, food products are frequently held for long periods and stored under adverse conditions of temperature and humidity. Prepared cake mixes that are commercially available cannot withstand these rigorous and protracted storage conditions. Consequently, a development project was undertaken by the Quartermaster Food and Container Institute to investigate new ingredients and evolve methods for increasing the storage tolerance of prepared cake mixes.

[1] Manuscript received August 25, 1952. Presented at the Annual Meeting, May, 1951. This paper reports research undertaken at the Quartermaster Food and Container Institute for the Armed Forces and has been assigned number 394 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsements of the Department of Defense.

In conjunction with the Quartermaster Food and Container Institute development program, a study sponsored by the Department of Defense was conducted by the University of Minnesota to determine the moisture equilibria of the various prepared mix ingredients and mixes (6). Data obtained in this study indicated that deteriorative chemical changes, primarily in leavening chemicals, occurred less rapidly at lower moisture contents. These observations led to the consideration of the role of moisture in the deterioration of prepared cake mixes and of possible methods of reducing moisture content in prepared cake mixes. Since the flour employed in cake mixes is the principal moisture-bearing ingredient, it obviously followed that flour dehydration would facilitate preparation of samples of reduced moisture content. Patents issued prior to this work and to other investigators during the course of these studies confirmed the utility of this approach (5, 7, 9).

Initial attempts to dehydrate cake flour by exposure on trays in a hot-air oven were disappointing. When soft-wheat cake flour was dehydrated below 8% moisture by these means, the baking properties of the flour were severely damaged by heat. Arrangements were made with the Institute of Industrial Research, University of Denver, to dehydrate a sample of soft-wheat cake flour using a "flash" dehydration system which had been effective in post-drying powdered egg products. A preliminary exploratory drying experiment demonstrated that soft-wheat flour could be dehydrated to 3.0% moisture without any evidence of heat damage to the baking properties of the flour.

Preliminary and exploratory stability studies upon prepared cake mixes suggested that such mixes, compounded with commercially available powdered shortening products, displayed greater storage tolerance than identical mixes compounded with plastic shortening. To explore more thoroughly the possibility of increasing the stability of cake mixes by use of these products, two types of commercially available powdered shortening were used in the experiments. This paper presents data of test-baking studies and analyses of free fatty acid of prepared yellow cake mixes compounded with one cake flour at four different moisture contents and a plastic shortening and two types of commercial powdered shortening, after various intervals of storage at 100°F.

Materials and Methods

Ingredients Employed. The bleached soft-wheat cake flour employed in compounding the yellow cake mixes analyzed 11.7% moisture, 7.8% protein (N × 5.7), and 0.32% ash calculated on a 14%

moisture basis. A portion of this flour sample was used as the undehydrated control flour and the remainder of the sample was used in the "flash" dehydration experiments at the University of Denver. A schematic sketch of the "flash" dehydration system used to dry these flour samples is shown in Fig. 1.

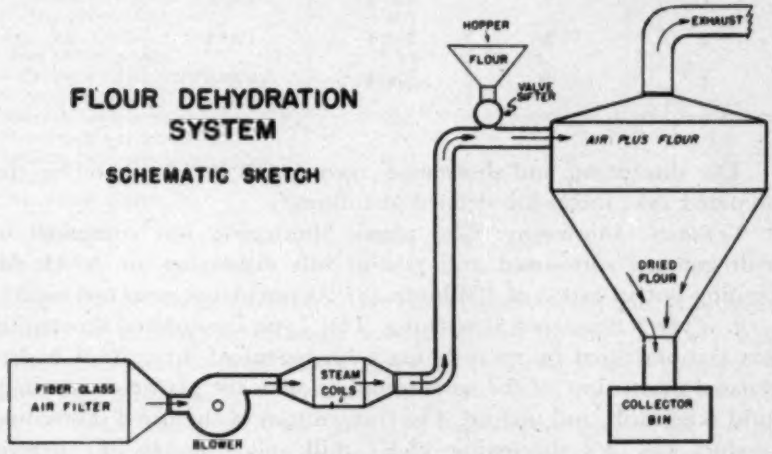

Fig. 1. Schematic diagram of flash dehydration system employed for drying flour.

In the operation of the system, incoming air is first filtered to remove air-borne foreign material and is then passed through the blower and over steam-heated coils. The flour to be dried is fed from the hopper through a sifter air-lock valve into the heated air stream. The mixture of flour and heated air then enters the cyclone collector. The dehydrated flour is discharged at the foot of the cyclone, and the moisture-laden air is exhausted at the top of the collector.

Dehydration runs were made to yield a series of three flour samples of dehydrated soft-wheat flours of various moisture contents. Seventy-five pounds of flour was dried for each sample. The operating conditions of the "flash" drier and the final flour moisture contents are shown in Table I.

Since the optimum drying conditions for flour and the operating limits of the system were unknown, the final moisture contents of the dehydrated flours could not be predicted. Undoubtedly, it would have been more desirable to have the moisture contents of the dehydrated flours more evenly spaced. Critical test-baking of the dehydrated flour samples in angel food and high-sugar layer cakes (140%) failed to show any evidence of heat damage to the flour.

TABLE I

FLOUR DRYING CONDITIONS

Sample Number	Number of Passes	Air-Stream Temperature	Flour Temperature When Collected	Final Moisture Content
				%
1	1	210°F.	136°F.	8.0
2	1	236°F.	157°F.	2.8
3	2	248°F.	178°F.	2.1

The shortening and shortening agents used in compounding the prepared cake mixes are defined as follows:

1. *Plastic Shortening.* The plastic shortening was composed of hydrogenated cottonseed and peanut oils displaying an A. O. M. keeping test in excess of 100 hours (2). Antioxidants were not used.

2. *Type I Powdered Shortening.* The Type I powdered shortening was manufactured by spray-drying a homogenized mixture of hydrogenated shortening (of the same composition as the plastic shortening), fluid skim milk, and sucrose. The composition of the dried shortening product was 73% shortening, 23.5% milk solids nonfat, 2% sucrose, and 1.5% moisture.

3. *Type II Powdered Shortening.* The Type II powdered shortening was manufactured by spray-drying a homogenized mixture of hydrogenated shortening (of the same composition as the plastic shortening), liquid egg yolks, fluid skim milk, and sucrose. The composition of the dried shortening product was 60% shortening, 18% egg yolk solids, 18% milk solids nonfat, 2% sucrose, and 2% moisture.

The powdered egg solids used in the cake mix formulation were not fermented and contained not more than 4% moisture. The powdered milk solids nonfat used were Premium Grade Spray Dried as defined by the Standards of the American Dry Milk Institute (1).

Cake Mix Formulation, Sample Preparation, and Storage. The formulas employed in compounding the prepared yellow cake mixes for the storage studies are illustrated in Table II.

A yellow cake mix was selected for the storage studies because it contained the fewest ingredients, and no flavoring ingredients which might mask off-flavors developed during storage. The three mix formulas were so balanced that the ratios of shortening, egg yolk solids, and milk solids nonfat were essentially the same for each formula. Each formula was compounded with an undehydrated control flour and the three dehydrated flours containing 8.0, 2.8, and 2.1% moisture respec-

TABLE II

YELLOW CAKE MIX FORMULAS

Ingredients	Formula 1	Formula 2	Formula 3
Granulated sugar (Baker's Special)	39.25	39.25	39.25
Plastic shortening	12.85	....	....
Egg-yolk solids	3.70	3.70	....
Milk solids nonfat	3.70	....	....
Type I powdered shortening	....	16.55	....
Type II powdered shortening	....	....	20.25
Sodium bicarbonate	0.55	0.55	0.55
Mono-calcium phosphate	0.37	0.37	0.37
Sodium acid pyrophosphate	0.37	0.37	0.37
Salt	1.00	1.00	1.00
Soft-wheat flour	38.21	38.21	38.21
Total	100.00	100.00	100.00

tively. In the prepared cake mixes compounded with the dehydrated flours, adjustments were made to compensate for the reduced moisture content of the flour. Thus, the flour solids were held constant in each mix formula.

Moisture analyses of the prepared cake mixes were made by the official A.O.A.C. vacuum-oven method (4). The relationships of flour moisture content to that of prepared mix are shown in Table III.

TABLE III

MOISTURE CONTENTS OF BLENDED CAKE MIXES

Flour Moisture Percentage	Nature of Shortening		
	Plastic	Type I Powdered	Type II Powdered
Control, undehydrated	4.3	4.4	4.3
8.0	3.3	3.1	3.0
2.8	1.2	1.4	1.2
2.1	0.9	1.0	0.9

Immediately after blending, 20-oz. portions of each mix were hermetically sealed in No. 2½ cans and stored in a constant-temperature room operated at 100°F. ± 2°.

Test-Baking and Analytical Methods. Samples were withdrawn from storage at monthly intervals for test-baking and moisture analyses. At bimonthly intervals free fatty acid content and peroxide values were determined upon fat extracted from the stored mixes.

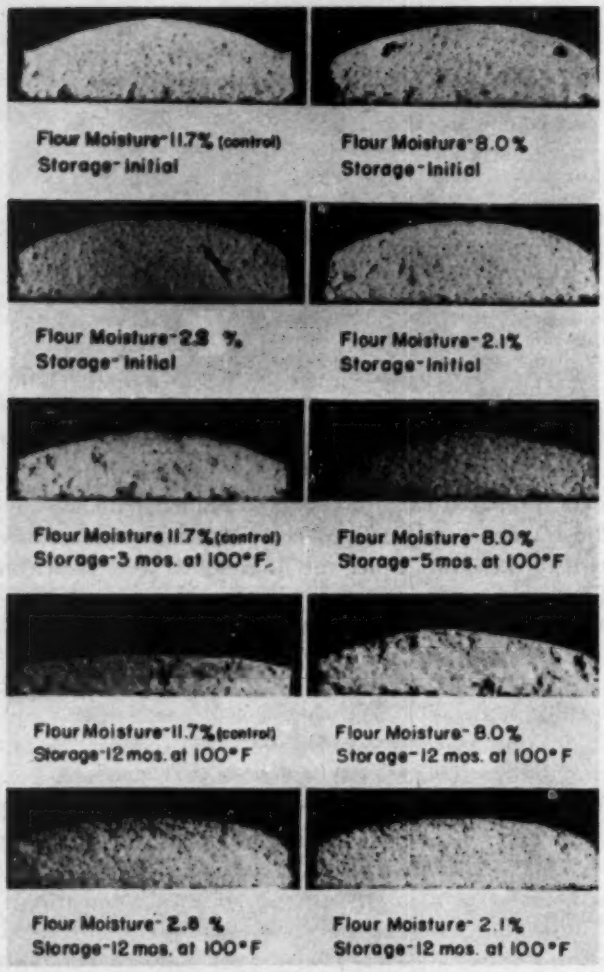

Fig. 2. Photograph of cakes baked from mixes made with plastic shortening, after storage for different periods.

For these determinations, the fat was extracted from the mix with chloroform at room temperature, using four successive extractions with 5 minutes of mechanical shaking for each extraction. The chloroform extracts were filtered through No. 12 Whatman filter paper, the filtrates combined and made up to a predetermined volume. Free fatty acid content (3) and peroxide values (8) were determined upon duplicate aliquots.

Cake batters for test baking were prepared, using 1 lb. mix and

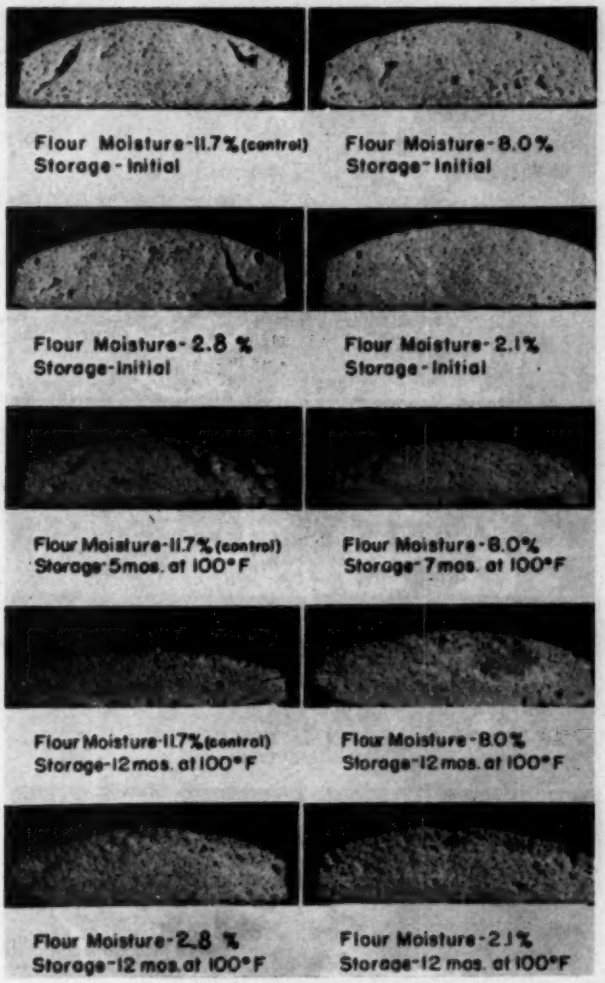

Fig. 3. Photograph of cakes baked from mixes made with powdered shortening (Type I), after storage for different periods. Type I powdered shortening contained 73 percent shortening, 23.5 percent milk solids nonfat, 2 percent sucrose, and 1.5 percent moisture.

8 oz. cold water. Adjustments were made in the batter recipe for those mixes compounded with dehydrated flours so that the moisture contents of the batter were held constant. Water was added in a three-stage procedure (50-25-25%). The batters were mixed in a 3-qt. bowl with flat paddle on a Hobart C-10 mixer in a standardized procedure on low speed. Two hundred and fifty grams of batter was baked in each of two 5-in. round layer tins at 350°F. for 25 minutes. After cool-

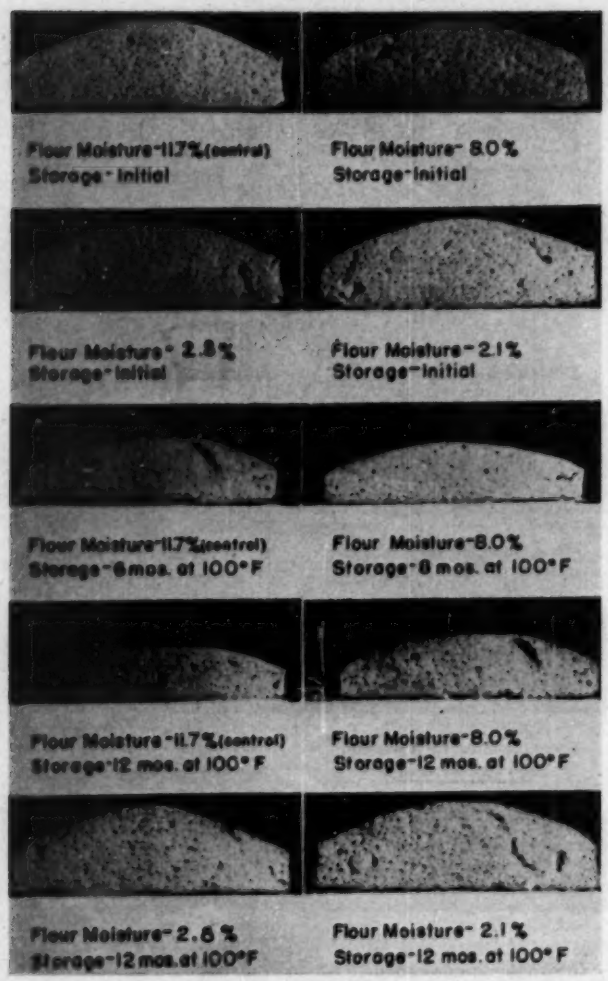

Fig. 4. Photograph of cakes baked from mixes made with powdered shortening (Type II), after storage for different periods. Type II powdered shortening contained 60 percent shortening, 18 percent egg-yolk solids, 18 percent milk solids nonfat, 2 percent sucrose, and 2 percent moisture.

ing to room temperature, cake volumes were measured by rape seed displacement.

Results and Discussion

Figures 2, 3, and 4 are photographs of cakes baked from the storage samples of prepared cake mix. The horizontal center row in each illustration shows cakes at the time they fell below the arbitrarily

established minimum levels in cake volume. Storage deterioration in the baked cake is characterized by loss of cake volume, indistinct grain structure, excessively tender and gummy texture, crumb discoloration, and objectionable off-flavor and aromas. The open-grain structure of the cakes compounded with powdered shortening (Figs. 3 and 4) is attributable to the poorer baking properties of the powdered shortening products used in this work.

The relationship of storage time to the specific volume (cake volume in cubic centimeters divided by cake weight in grams) of cakes baked from the stored mixes is shown in Fig. 5, which consists of three graphs arranged according to the shortening or shortening agent employed. The horizontal bars at specific volume 2.25 indicate the arbitrarily established minimum acceptable specific volume.

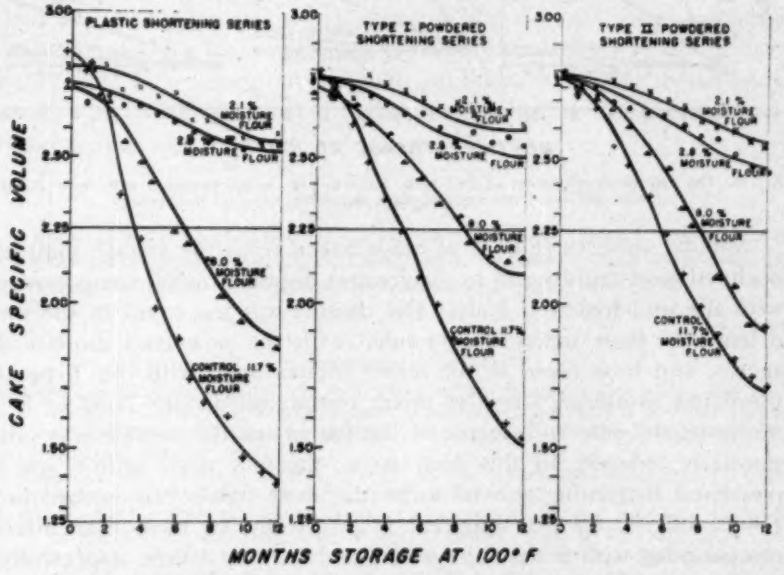

Fig. 5. The relation of storage time to the specific volume of cakes baked from prepared mixes compounded with flour of different moisture contents and with three types of shortening.

The rates at which free fatty acids develop in the prepared cake mixes during storage are shown in Fig. 6.

Peroxide values determined upon the chloroform-extracted fat from the stored mixes showed no increase in the majority of the analyses. In those few instances where increases were indicated, no correlation with storage time was apparent. The aromas of the stored mixes and cakes baked from the mixes were not characteristic of oxidative rancidity in any instance.

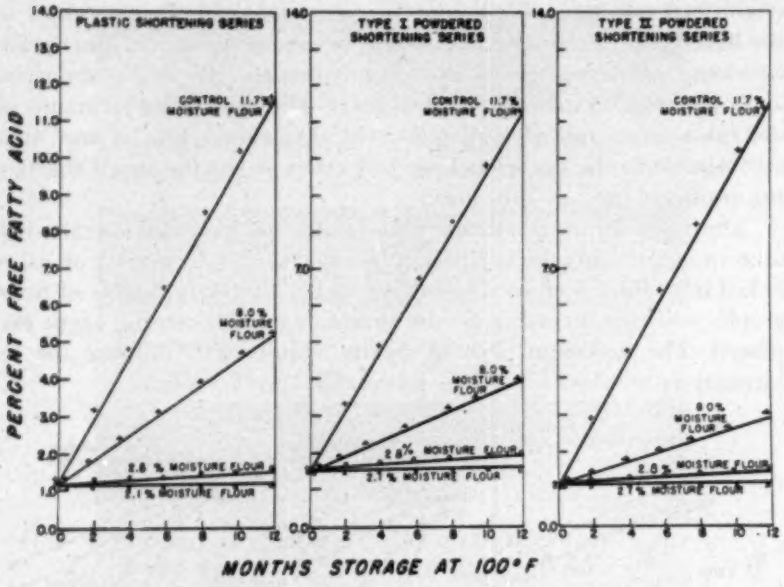
PLASTIC SHORTENING SERIES
TYPE I POWDERED SHORTENING SERIES
TYPE II POWDERED SHORTENING SERIES
CONTROL 11.7% MOISTURE FLOUR
8.0% MOISTURE FLOUR
2.8 % MOISTURE FLOUR
2.1 % MOISTURE FLOUR
PERCENT FREE FATTY ACID
MONTHS STORAGE AT 100°F

Fig. 6. The rate of development of free fatty acids in cake mixes prepared with flour at different moisture contents and with the three types of shortening.

Specific volumes (Fig. 5) of cakes baked from the storage samples declined most rapidly and to the greatest degree in mixes compounded with the undehydrated flours. The decline was less rapid in the undehydrated flour mixes compounded with the powdered shortening agents, and least rapid in the mixes compounded with the Type II powdered shortening. In the mixes compounded with flour at 8% moisture, the rate and degree of decline in specific volume was substantially reduced. In this flour series, the mix made with Type I powdered shortening proved to be the most stable. No explanation can be offered for this apparent inconsistency. In those cake mixes compounded with flours dried to 2.8 and 2.1% moisture, respectively, there was a slight decline in cake specific volume over the 12-month storage period. However, these cake specific volumes all remained well above the arbitrarily established limits for cake volume throughout the storage period. At what time these mixes would have fallen into the unacceptable range must remain a matter of speculation.

Development of free fatty acid (Fig. 6) proved to be a straight-line function of time of storage. In the mixes compounded with the undehydrated flour, free fatty acids developed at a constant rate regardless of the shortening or shortening agent employed. However, in the mixes compounded with the flour containing 8% moisture, differences

in the rate of development of free fatty acid did appear; development was most rapid in the plastic shortening mix and least rapid in the Type II powdered shortening mix, and in those mixes compounded with the flours at 2.8 and 2.1% moisture it proceeded at an extremely low rate or was arrested entirely.

The high free fatty acid content of cake mixes of deteriorated baking properties certainly suggests that development of free fatty acid is associated with deterioration. While development of free fatty acid parallels deterioration of cake mix in most instances in these experiments, a direct correlation between free fatty acid content and degree of cake mix deterioration is not apparent. Moreover, in these experiments it is uncertain whether the fat splitting is the result of lipase activity or chemical hydrolysis.

These experiments demonstrate that the use of dehydrated cake flour is an effective means of extending the storage life of prepared cake mixes. Information is lacking, however, on the stability of prepared cake mixes compounded with cake flours ranging in moisture content from 8.0 down to 2.8%. Experiments to provide this information are in progress.

Acknowledgments

Appreciation is expressed to Dr. George O. Löf and Mr. Dent C. Davis, Jr., of the Institute of Industrial Research, University of Denver, for their cooperation in conducting the "flash" dehydration experiments on the flour used in these experiments.

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COLORIMETRIC AND FLUOROMETRIC PROPERTIES OF WHEAT IN RELATION TO GERM DAMAGE[1]

EARL W. COLE AND MAX MILNER

ABSTRACT

Recognition of germ-damaged or "sick" wheat by commercial inspection procedures involves visual detection of a brown to black discoloration in the germ. Small but significant differences in the optical density of extracts of commercial germ-damaged and normal grains were found in the ultraviolet region centering around 250 to 325 mμ. A great intensification of these absorption differences appeared on analysis of germs dissected from this grain. The same absorption spectra were yielded by whole commercial wheat germ which had browned in laboratory storage at moderately elevated temperature and moisture content. These results suggest that this discoloration associated with germ-damaged or "sick" wheat is due to a browning reaction of the Maillard type localized in the germ.

Fluorescence was found to be more sensitive than colorimetry for differentiating sound from germ-damaged wheat and in revealing the progressive browning in stored germ. Nevertheless, absorption and fluorescence showed a linear relationship throughout the range of values found. Differences in fluorescence were shown not to be associated with changes in riboflavin concentration.

The fluorescence of numerous samples of sound wheat widely different in class, variety, composition, and source shows remarkable uniformity, probably indicative of fluorescence due to the non-germ portion of the grain. A statistically significant positive correlation was found to exist between commercial graders' evaluation of sick wheat and the fluorescence value, which suggests the feasibility of the method for objective evaluation of germ damage in wheat.

The deterioration in commercial wheat designated by federal and licensed grain inspectors as germ-damaged, dark-germ, or "sick" is characterized by a tan, brown, or black discoloration in the embryo which becomes apparent when the bran layer above the germ is scraped off. This condition appears to be associated with elevated fat acidity and the appearance on the embryo of numerous fungi, principally of the species *A. flavus* and *A. glaucus* (6). These microorganisms are known to secrete toxic materials which reduce the germination of the grain (16). The discoloration of the germ, however, is the principal factor upon which commercial detection is based.

The precise cause of the "sick" wheat condition is not altogether clear, although it is associated with wet harvest seasons and the storage

[1] Manuscript received March 23, 1953. Contribution No. 235, Department of Flour and Feed Milling Industries, Kansas State College, Manhattan. Presented at the Annual Meeting of the American Association of Cereal Chemists, Buffalo, N. Y., May, 1953.

This research is a portion of a thesis presented by Earl W. Cole as partial fulfillment of the requirements for the Degree of Master of Science in Milling Industries at Kansas State College.

of grain at elevated moisture values and temperatures, conditions which also favor the development of fungi (6). There is evidence, however, that the dark germ condition may appear with storage at low moisture values where fungal growth probably cannot occur (1), or under conditions where fungal growth is inhibited by treatment with chemical preservatives (5, 14). No information exists regarding the nature or importance of the characteristic brown discoloration of the germ. It has been suggested (6) that this darkening may be due to a browning reaction of the Maillard type involving condensation of reducing sugars and proteins (4). Such browning reactions are well known in other stored foodstuffs of low moisture content such as dried fruits (13), dried eggs (8), and dried powdered milk (11, 15). This hypothesis provided the incentive for the present study of the characterization by colorimetric and fluorometric technics of extracts of commercial germ-damaged wheat and of wheat germ browned in laboratory storage. The utility of such methods for determination of the extent of germ damage in commercial grain samples was also investigated.

Materials and Methods

About 50 samples of commercial wheat showing various degrees of germ damage as determined by federal grain inspectors were acquired from the Production and Marketing Administration, U. S. Department of Agriculture, Kansas City, Missouri. The fresh, unprocessed, granular wheat germ was obtained from commercial sources and was stored in the cold room until used. Ninety-one sound samples of wheat representing numerous commercial classes and varieties were obtained from the following sources:

Class	*Location*
Soft red winter (10 varieties)	Wooster, Ohio
Pacific Coast wheat (13 varieties)	Lind and Pullman, Wash.
Hard red spring (2 varieties)	Langdon Substation, N. D.
Hard red winter (66 samples comprising 6 varieties from 11 locations)	Kansas State College Agronomy Farm, Manhattan, and Branch Experiment Stations in Kansas

Preparation of clear extracts of germ-damaged wheat and darkened germ suitable for colorimetric and fluorometric analysis was investigated by using various solvents including 95% ethanol, water-saturated butanol, 2.5% aqueous potassium hydroxide, acetone, dioxane, and other nonpolar solvents. None of these was as good as

water alone. pH values above 7 were associated with the appearance of a yellow color in the extract due to a flavone pigment which caused erratic differences in optical density apparently not related to the browning characteristic of the germ-damaged condition. For this reason an aqueous medium of low pH (0.2*N* hydrochloric acid) was used. The very low pH also minimized the solubility of proteins, thus yielding clearer extracts, and at the same time suppressed the fluorescence of riboflavin which might otherwise have interfered with the measurements.

The procedure finally adopted was as follows: The wheat was ground to pass through the No. 30 screen of the intermediate Wiley mill. A 5-g. sample of wheat or 1 g. of wheat germ was extracted for 1 hour in a stoppered Erlenmeyer flask with 100 ml. of 0.2*N* hydrochloric acid, with swirling about once every 15 minutes. The material was centrifuged for 5 minutes at 1,500 r.p.m. and then decanted. Clarification of extract was accomplished by adding 5 ml. of chloroform and shaking vigorously for 1 minute in order to precipitate the suspended materials. The utility of this reagent for the precipitation of proteins was suggested by the work of Sevag *et al.* (12). The material was centrifuged again for 5 minutes and the clear supernatant was analyzed. Necessary dilutions were made with 0.2*N* hydrochloric acid.

Optical absorption measurements of these extracts were carried out with the Beckman Model DU Spectrophotometer in the region of 220 to 350mμ, using quartz curvettes. Fluorescence measurements were made on the same extract with the Coleman photoelectric fluorometer with Vitamin B_1–S and B_2 filters. Sodium fluorescein (0.1 p.p.m.) was used to standardize this instrument, the dial being set at 60 with this solution. With fluorescence measurements, no dilution of the extract was necessary with the B_2 filters, but with the B_1–S filter 1 ml. of extract was diluted to 50 ml.

The determination of riboflavin was carried out by the procedure of Hoffer *et al.* (2), and the method described in Cereal Laboratory Methods was used for thiamine.

Results

Optical Characteristics of Extracts of Sound and Germ-Damaged Wheat. The aqueous acid extracts of a sound commercial wheat sample and of a similar sample containing 50% of germ damage were found to have significant absorption in the ultraviolet and considerably less absorption in the visible spectrum. Also, as indicated in

Fig. 1, the extracts of the germ-damaged grain showed slightly higher optical density values between 240 and 265 mμ and between 295 and

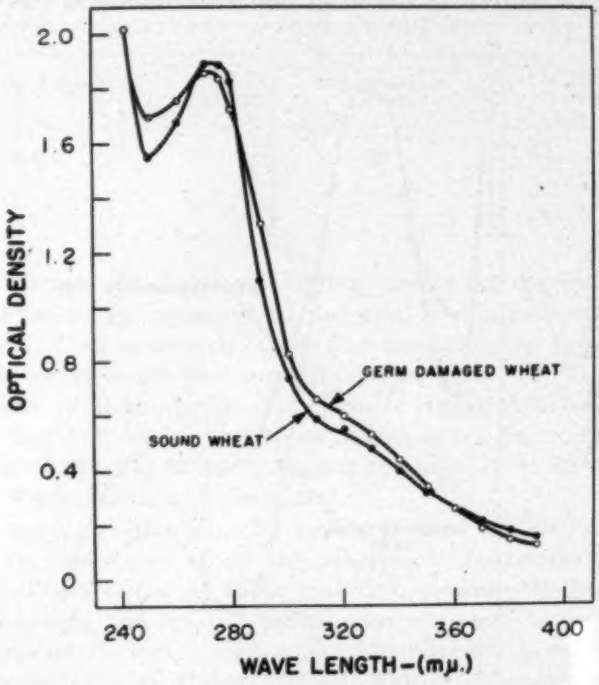

Fig. 1. Absorption spectra of extracts of sound and germ-damaged wheat.

350 mμ than did the sound grain. While these differences were not great, absorption in this same spectral region is characteristic of browning reactions in model systems containing pure proteins and reducing sugars (7).

Spectrophotometric and Fluorometric Characteristics of Germ and Non-Germ Portions of Sick and Sound Kernels. To determine the location and distribution of the brown color in the various tissues of sick wheat which appeared to be associated with the spectrophotometric and fluorometric properties of the extracts, a study of the dissected fractions of damaged and sound kernels was carried out. Six grams each of apparently sound kernels and germ-damaged kernels were picked out from a commercial sample grading 60% germ-damaged. The germ portions of all the kernels were carefully dissected and segregated. The two fractions were then ground and analyzed in the usual way, care being taken to maintain the solvent ratio uniform for all samples.

The spectral curves for the extracts of dark germ and of sound germ obtained from the commercial germ-damaged grain are shown in Fig. 2. In contrast to the small differences found in the spectral

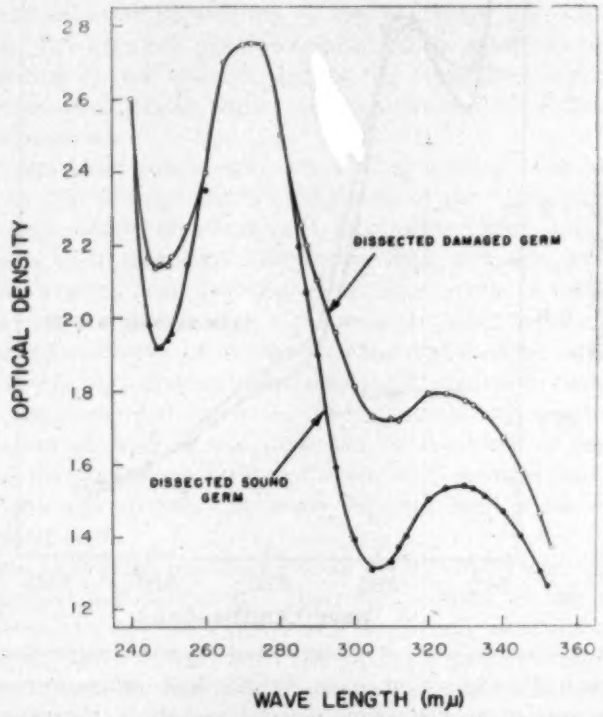

Fig. 2. Absorption spectra of extracts of germ dissected from damaged and sound kernels from the same commercial sample.

characteristics of normal and germ-damaged whole grain, a decided increase in absorption in the same critical spectral regions occurs when the germs alone are analyzed. These results indicate that the major optical changes noted occur in the germ portion of the grain. This is further confirmed by the data of Table I which indicate the fluorescence characteristics of the dissected fractions.

The increased fluorescence which accompanies the sick condition is due almost entirely to changes in the germ, and any changes in the endosperm associated with the germ-damaged condition as indicated by fluorescence are relatively minor. It seemed probable that the brown pigmentation in "sick" germ is responsible for both colorimetric and fluorometric characteristics described.

Changes in Stored Wheat Germ Related to Germ Damage in

TABLE I

FLUORESCENCE OF EXTRACTS OF EQUAL WEIGHTS OF DISSECTED GERM AND NON-GERM PORTIONS OF DAMAGED AND APPARENTLY SOUND KERNELS FROM THE SAME SAMPLE

Portion of Kernel	Fluorescence	
	Germ-Damaged Wheat	Sound Wheat
Germ	132.4	25.6
Non-germ	6.9	3.9

Wheat Kernels. The previous findings prompted the use of fresh wheat germ as an experimental material. Preliminary studies had shown that fresh commercial germ also would darken rapidly when stored at elevated moisture and temperature values.

Portions of fresh commercial granular wheat germ conditioned to 12% and 17% moisture content respectively were stored at temperatures of 25° and 38°C. At regular intervals up to 42 days, samples were withdrawn and analyzed.

The samples containing 12% moisture stored at 25°C. showed no change in appearance or in the absorption characteristics of the extracts. Those stored at 38°C. and 12% moisture showed only a slight browning and spectral analysis was not made.

Samples of the germ containing 17% moisture stored at 38°C., when examined after 4 days, showed noticeable browning. Intense browning existed by the 25th day of storage. Mold growth became noticeable on the 35th day. Considerably less color change occurred in the sample stored at 12% moisture content at 38°C. than in the sample at 17% moisture content stored at 25°C. The sample with 12% moisture content stored at 25°C. showed no apparent change in color.

Spectral properties of extracts of the germ containing 17% moisture stored at 38°C. after 11, 25, and 32 days of storage appear in Fig. 3. These curves follow closely those previously obtained with browned germ dissected from commercial sick-wheat grains. The same progressive increase in absorption around 250 and 325 $m\mu$ appears in this stored brown germ. A fluorescence measurement made on this sample after 2 months of storage yielded a value of 800 units as compared with a value of 2 for fresh sound granular germ.

Relationship of Fluorescence to Optical Density in Extracts of Browned Germ. The question whether the pigment in the wheat extracts which causes fluorescence and that responsible for the ultra-

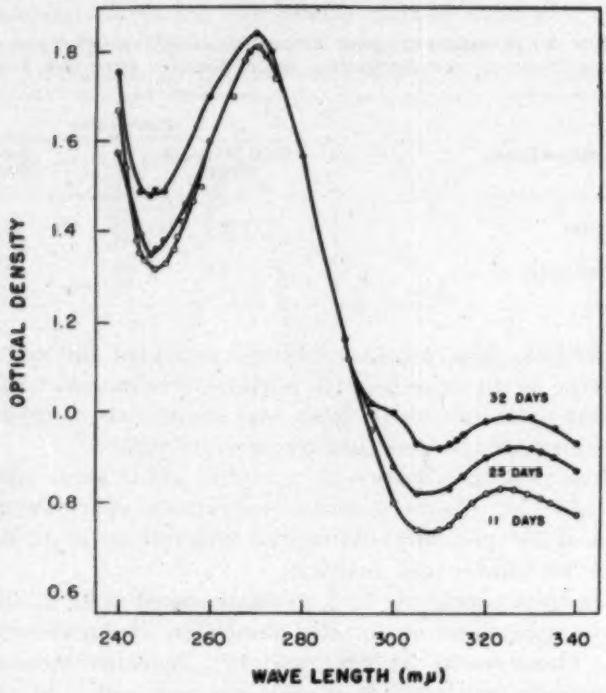

Fig. 3. Absorption spectra of extracts of wheat germ stored at 17% moisture and 38°C. for various periods of time.

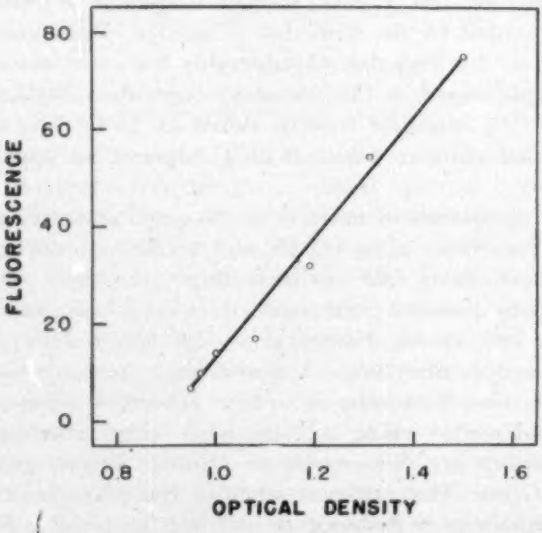

Fig. 4. Relationship of fluorescence to optical density of extracts of browned germ.

violet absorption characteristics are the same was investigated by recording periodically fluorescence values and optical densities at 225 $m\mu$ of samples of the granular germ stored at 38°C. and 17% moisture as the browning progressed. The results are shown in Fig. 4, and indicate that a linear relationship exists between the two factors. This suggests that fluorescence and absorption are due to the same brown pigment in wheat germ which increases progressively with deterioration.

Negative Role of Riboflavin in the Fluorescence of Browned Germ Extracts. During a study of the preparation of extracts of germ-damaged wheat for colorimetric and fluorometric analyses it was observed that the change in fluorescence in wheat extract with change in pH appeared to be virtually identical with the fluorescence curve of riboflavin at various pH values, as had been noted by Karrer and Fritzsche (3). While any fluorescence due to riboflavin was minimized by the low pH of the extraction medium used in this work, it appeared of interest, nevertheless, to determine whether the increase in fluorescence which occurs upon the browning of wheat germ might be related to changes in concentration of riboflavin.

Samples of the germ showing browning due to storage at 18% moisture and 38°C. were analyzed for fluorescence and for riboflavin content. The data, given in Table II, show that the marked

TABLE II

FLUORESCENCE AND RIBOFLAVIN CONTENT OF WHEAT GERM AT VARIOUS STAGES OF BROWNING

Storage Time	Fluorescence of extract	Riboflavin
days	*scale units*	*μg./g. dry wt.*
17	28	5.5
25	42	4.6
32	57	5.0

increase in fluorescence of the aqueous acid extracts which is characteristic of browning in wheat germ is not accompanied by a significant change in riboflavin concentration. Accordingly, it appears that this vitamin is not involved in the fluorescence phenomenon of interest in this work. Analysis for thiamine showed that there was also no change in concentration of this vitamin with germ deterioration, which confirms the results of previous studies (9).

TABLE III

RELATIONSHIP OF SOURCE, CLASS, VARIETY, AND COMPOSITION TO FLUORESCENCE VALUE OF WHEAT SAMPLES

Number of Individual or Composited Varieties from Given Location	Date of Harvest	Protein			Ash			Test Weight			Fluorescence		
		Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean
Soft red winter composites (Wooster, Ohio)		%	%	%	%	%	%						
10	1947	11.3	9.3	10.22	1.82	1.67	1.74	61.7	58.5	59.9	30.0	24.5	26.5
Pacific Coast composites (Lind and Pullman, Wash.)													
13	1949	13.1	10.5	11.5	1.63	1.42	1.49	67.4	60.0	63.0	27.8	24.3	26.1
Hard red spring individual samples (North Dakota)													
2	1949	...	...	15.6	...	...	...	...	...	61.9	...	...	27.4
Hard red winter individual samples (Kansas)													
3 (Manhattan)	1952	11.4	11.0	11.2	1.79	1.57	1.69	62.1	60.2	61.2	23.7	22.5	23.2
5 (Manhattan)	1952	11.1	9.9	10.6	1.76	1.65	1.70	63.0	61.0	62.2	31.0	27.3	29.8
6 (Tribune)	1952	17.0	15.7	16.5	1.80	1.66	1.73	59.0	53.8	55.8	33.5	30.0	32.0
6 (Kingman)	1952	13.7	12.6	13.0	1.51	1.34	1.42	64.0	60.5	62.2	29.0	25.5	27.5
6 (Belleville)	1952	11.2	10.8	11.0	1.91	1.82	1.85	64.2	61.0	62.5	31.5	27.0	29.5
6 (Hays)	1952	13.6	12.1	12.7	1.70	1.59	1.64	63.2	60.2	61.4	35.0	30.5	32.2
6 (Hutchinson)	1952	11.7	10.0	11.0	1.61	1.50	1.53	64.5	60.6	62.6	32.3	30.2	31.3
6 (Dodge)	1952	15.9	14.9	15.3	1.64	1.52	1.57	63.2	60.4	61.8	33.2	25.5	29.4
6 (Garden City)	1952	13.4	11.3	12.3	1.57	1.44	1.51	61.5	59.1	60.2	31.1	27.0	29.2
6 (Colby)	1952	14.2	12.2	13.0	1.90	1.71	1.78	61.0	58.0	59.5	32.5	29.0	30.5
6 (Mankato)	1952	16.8	12.3	15.1	1.82	1.60	1.71	59.0	53.5	55.8	30.7	24.5	28.3
5 (Thayer)	1952	9.0	8.8	8.9	1.64	1.51	1.58	63.5	60.0	61.7	31.0	30.0	30.3

Influence of Source, Class, Variety, and Composition of Sound Wheat on Fluorescence Value. The apparent increase in fluorescence accompanying deterioration in commercial germ-damaged samples suggested that this characteristic might provide an objective method for the quantitative evaluation of germ deterioration in commercial grain. Before such a conclusion could be reached, it appeared necessary to determine the variability in fluorescence of sound grain and particularly any differences in this factor in wheats as related to class, variety, agronomic background, environmental factors, and composition.

The results of fluorescence analyses of 91 samples of sound wheat of widely differing characteristics are summarized in Table III. Nor-

TABLE IV

FLUORESCENCE OF 0.2*N* HYDROCHLORIC ACID EXTRACTS AND VIABILITY OF GERM-DAMAGED WHEAT GRADED BY FEDERAL GRAIN INSPECTORS

Graded Damage	Fluorescence	Germination
%		%
5.0	34.0	78
7.0	37.7	83
9.0	34.0	48
12.0	39.4	50
15.0	50.5	63
16.5	50.0	57
17.0	38.4	34
17.0	36.2	39
18.0	43.0	37
20.0	40.1	56
22.0	42.0	55
22.0	30.0	63
24.0	48.8	65
25.0	60.7	27
26.0	50.0	44
28.0	51.0	31
36.0	62.0	0
40.0	56.0	16
40.0	42.7	32
50.0	61.0	13
63.0	54.0	10
64.0	61.5	0
68.0	55.5	4
70.0	58.0	8
76.0	62.0	1
80.0	59.7	17

mal or sound wheats, differing widely in those characteristics which affect their nature and quality, show a surprisingly narrow and uniform range of fluorescence which does not appear to be affected by test weight, protein content, or ash content. These results suggest

that the fluorescence test for the browning associated with germ damage is essentially independent of the major compositional, environmental, and genetic factors which normally are highly variable in wheat.

Correlation of the Fluorescence Test with Commercial Grain Inspectors' Evaluation of Germ Damage. To evaluate the relation between the fluorescence and the extent of germ damage, as determined by grain graders, fluorescence tests were made on 26 samples of commercial wheat ranging in graded damage from 5% to 80% which were supplied by the federal grain inspection office in Kansas City. Germination tests also were made by the Kansas State Seed Testing Laboratory. The results of this study appear in Table IV. The correlation coefficient of 0.748 for fluorescence *versus* graded germ damage and 0.775 for fluorescence *versus* germination are significant at the 1% level.

Discussion

The pronounced fluorometric and colorimetric changes related to the development of brown discoloration in commercial sick wheat and in wheat germ stored at elevated moisture and temperature provide strong evidence that a browning reaction of the Maillard type may be a fundamental cause of germ damage in wheat. Fluorescence measurement for characterizing the browning reaction in other food products is an established technic (8, 10). Mohammed *et al.* (7) have employed spectrophotometry to follow the color development in browning reactions and the characteristics of such absorption spectra are similar to those which were obtained with extracts of germ-damaged grain in the present study. Evidence from experimental studies now in progress in this laboratory is providing additional support for the contention that a nonbiological browning reaction is the principal cause of discoloration in germ-damaged wheat.

The role of microorganisms in the development of the germ-damaged condition was not specifically investigated in this study. The activity of fungi in relation to the brown discoloration remains obscure, since such colors were readily obtainable in germ stored at temperatures where fungi cannot be expected to grow. Furthermore, at elevated moisture contents and at temperatures where fungi can proliferate, the appearance of the brown color precedes the appearance of fungi. It is conceivable, nevertheless, that grain in which mold growth has occurred may develop germ damage more readily than will grain in which there has been no preliminary mold activity. Doubtless, the increase in temperature which favors germ discolora-

tion in ordinary storage is due to the thermogenic activity of fungi in the grain. However, the suggestion that the chemical products of mold metabolism provide the precursors for the subsequent browning phenomenon remains to be verified.

The small differences in optical density between sound and damaged kernels in critical regions of the absorption curves is a reflection of the fact that the deterioration is confined almost entirely to the germ, which constitutes only from 1 to 5% of the kernel. It appears that only after the germ damage becomes well advanced does the brown coloration in the germ invade the endosperm, and this may be due to leaching of the water-soluble brown pigment. The deleterious effects of germ damage in wheat on the baking properties of flour obtained from such grain have been described in the literature (5, 14). The effects noted may be caused by damaged germ material incorporated into the flour during milling rather than by some specific deterioration in the endosperm. The germ of sick wheat is more brittle and fragile and thus more of it would appear in the flour.

The identical colorimetric and fluorometric characteristics of brown germ dissected from commercial samples of sick wheat with those of germ browned by storage in the laboratory indicate that the latter material is ideal for experimental study of the sick-wheat phenomenon. It is clear that the germ of wheat is much more sensitive to deterioration than has been appreciated heretofore. That wheat germ is very labile, particularly at moderately elevated temperatures and moisture values, was indicated by Pearce (9) and confirmed by the present studies. This lends credence to reports from the grain trade concerning the extreme rapidity with which sick wheat can develop in storage.

Preliminary chromatographic studies in this laboratory of the aqueous acid extract indicate the presence of two components, a yellow flavone pigment and a dark brown material. The increase in concentration of the latter appears to be the cause of the intensification in the spectral and fluorometric characteristics found in this study, although its chemical nature remains to be determined.

The low and virtually uniform fluorescence of sound wheat samples of widely different class, source, variety, and composition, as well as the insignificant fluorescence observed in fresh granular wheat germ, is taken to mean that the fluorescence in sound grain is due almost entirely to the endosperm or at least the non-germ portion, and that increases in fluorescence appear with the brown color associated with germ damage.

Favorable indications have been obtained in this study that, with additional refinement and simplification, the fluorescence test may be useful for evaluating quantitatively the sick or germ-damaged content of commercial wheat samples. The test also appeared to be useful as an indication of the viability of such grain. The correlation coefficient of 0.748 between the fluorescence test and the commercial grader's evaluation of the sample appears good in view of the highly subjective manner in which such grain is graded. Whereas the fluorescence test provides an overall evaluation, combining the quantity and intensity factors of germ browning, the commercial inspection technic takes into account only the fact that a germ is discolored, and no attention is given to the degree or intensity of the coloring or deterioration, i.e., whether the germ is light tan, brown, "mahogany," or black. For this reason the correlation which was obtained with the fluorescence test must be considered good. One difficulty with the fluorescence determination as now used is the rather narrow range of analytical values encountered, from about 30 for sound wheat to about 60 for severely damaged grain. This precludes the indication of small differences in damage and germination in the kernels. The basic problem lies in the fact that fluorescence changes occur primarily in the germ portion, which constitutes only from 1 to 5% of the grain. While dissection of the germs from the kernels would permit more accurate analysis of this tissue, such a step would appear to increase rather than decrease the complexity of the test. A more useful refinement would be a means to minimize the fluorescence of the endosperm to permit more accurate differentiation of small degrees of germ browning.

Acknowledgment

The authors wish to thank Mr. J. E. Elstner, Production and Marketing Administration, U. S. Department of Agriculture, Kansas City, Missouri, for the series of graded commercial samples. Dr. F. C. Hildebrand, General Mills, Inc., kindly supplied the commercial wheat germ.

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DECOMPOSITION OF BROMATE IN DOUGH[1]

I. HLYNKA, P. R. TEMPLIN, AND J. A. ANDERSON

ABSTRACT

The action of potassium bromate as an oxidizing agent in flour-water dough, straight dough and sponge dough, was studied by following loss of bromate. For flour-water dough, comparative changes in physical properties (extensograms) were also determined.

Little or no loss of bromate was demonstrated in flour-water doughs containing 10 and 30 p.p.m., mixed up to 8 minutes, which were allowed a reaction time and rest period up to 3 hours, rounded in an extensograph rounder, and remixed; previous work showed significant changes in extensograms for doughs similarly treated. Bromate loss increased with decreasing flour quality; extensograms showed no clear trends with flour quality. For a given flour bromate loss was found to be the same at 30 and 60 p.p.m.; increase in extensogram height was about 2.5 times as large at the higher level. Addition of lactic and hydrochloric acid increased bromate loss only very slightly; but increase in extensogram height was pronounced under the same conditions.

Yeast was found to accelerate the decomposition of bromate in dough. Definite reduction was shown at various stages of the test baking procedure. In experiments lasting 15 to 20 hours, no bromate could be recovered. When bromate was added at the doughing up stage, no evidence for the

[1] Manuscript received April 7, 1953.
Paper No. 128 of the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, Manitoba, and No. 308 of the Associate Committee on Grain Research (Canada).

accumulation of reducing metabolic products of yeast could be obtained. Some bromate loss may be attributed to microbiological activity in flour-water doughs in experiments of long duration.

On basis of data obtained, it was not possible to identify the mechanism of decomposition of bromate in dough with the mechanism of improver action.

The present investigation deals with the course of decomposition of potassium bromate in dough, and with the question of whether or not the decomposition is associated with certain changes in physical properties of dough and with improver action in bread.

References in the literature to the fate of bromate in dough are not many. Sullivan, Howe, Schmalz, and Astleford (12) reported that over half of 10 p.p.m. added bromate was used up immediately upon mixing the dough, and that a comparatively small additional amount was used up during a subsequent 3-hour fermentation. In analogous experiments with potassium iodate containing radioactive iodine, Conn, Hollenbeck, Rosenblum, and Woodbury (3) showed that the major decomposition product of potassium iodate in bread was potassium iodide. Working with another analogous substance Baker, Parker, and Mize (2) found that sodium chlorite completely disappeared in gluten-containing fractions of dough. Finally, Auerbach, Eckert, and Angell (1) reported that 1000 p.p.m. of persulfate incorporated in dough disappeared completely in 3.5 hours.

Interpretation of the mechanism by which bromate disappears from dough has been limited to observations by several investigators (5, 10, 12, 13) that reducing substances of flour or gluten, or those produced by yeast in fermenting doughs react with some of the bromate. In addition there is an extensive literature on hypotheses which have been proposed to explain the action of bromate as a bread improver. An objective summary has been published by Hildebrand (6).

This paper describes two related series of experiments. The first series was designed to determine whether changes in the physical properties of bromated doughs as measured by the extensograph are accompanied by a parallel oxidizing action of bromate determined analytically as bromate loss. These experiments were carried out on flour-water doughs. The second series of experiments was made with fermented doughs and was designed to assess the influence of yeast activity on bromate decomposition. The experiments included both short-time studies corresponding to pre-oven stages of a straight-dough baking test, and straight and sponge dough fermented for longer periods up to 20 hours. Details of the experimental methods and the results obtained in this investigation are presented and discussed.

Materials and Methods

The method for the determination of bromate was that of Johnson and Alcock (9) adapted for dough. In principle the method consists of preparing a potassium chloride extract of dough, reacting the bromate in the extract with potassium iodide to yield free iodine and developing a blue color with a starch reagent. The optical density of the resulting solution was measured with a Beckman spectrophotometer at 575 mμ. The method does not give results of as good precision for dough as it does for flour. For dough the error is about ±6% and interpretation of the results must be made within these limits. In one set of experiments Howe's method (8) for bromate determination was used.

Several different experimentally milled flours were used during the course of the investigation. The particulars of each flour are listed in Table I. Flours A, C, D, and E were straight grade, and were milled to an extraction of about 70%. Flour B was milled from one parcel of wheat and separated into first patent 40%, second patent 27%, and clear 6% to give flours of decreasing quality.

TABLE I

FLOUR USED IN INVESTIGATION

Flour	Wheat Grade	Flour Protein	Farinograph Absorption
		%	%
A straight	1 & 2 Northern	13.1	64.7
B 1st patent	1 & 2 Northern	11.6	59.0
B 2nd patent	1 & 2 Northern	13.4	62.0
B clear	1 & 2 Northern	14.4	65.4
C straight	1 Northern	12.2	61.0
D straight	3 Northern	13.1	60.8
E straight	2 & 3 Northern	13.0	62.6

Flour-water doughs and yeast doughs were prepared incorporating the amount of bromate required. For yeasted straight doughs the regular malt-phosphate-bromate formula was used. For longer fermentation experiments, sugar was omitted from the formula. Sponge doughs were the same as straight doughs except that only 70% of flour and water was used. After the required fermentation of the sponge, the remainder of water was added and the dough mixed to a gruel-like consistency, in 1¼ minutes, then the remainder of the flour was added and the dough was mixed for a further ¾ minute. In these experiments bromate was introduced at the doughing up stage. All doughs

were rested or fermented in a cabinet controlled at a temperature of 30° C. and a relative humidity of 85%.

For the determination of bromate, a suitable sample, usually 7 g., was dispersed in a Waring Blendor for 1 minute in 40 ml. solution of potassium chloride and iodine, and the procedure as laid down by Johnson and Alcock (9) was followed. In calculation of the results, the amount of water contained in the dough was taken into account and the results were expressed on flour basis of 14% moisture.

Extensograms were obtained in the usual manner, initially after a rest period of 45 minutes after shaping the dough test pieces, and after allowing a reaction period of 135 minutes, then shaping and resting the dough for a further 45 minutes. No salt was used in the test doughs. All tests were conducted at 30°C.

Results

The design of the first part of the investigation relates to extensograph technique. There are several critical points in the procedure of testing dough for bromate on the extensograph. In work which has been recently published (4, 11), it was established that bromate has little effect on extensograms if doughs are shaped immediately after mixing irrespective of the "rest period" permitted between stretching and shaping. Bromate effect as exemplified by a higher and shorter extensogram, is produced only if the dough is shaped after a reaction time and is a function of reaction time. A "rest period" is usually permitted between the final shaping and stretching. As this rest period is extended, up to about 45 minutes, the difference between extensograms for unbromated and bromated doughs becomes approximately constant.

Table II deals with bromate recovery corresponding to various treatments of dough suggested by the foregoing stages of extensograph technique. Flour A was used throughout with 30 p.p.m. bromate. The first four experiments show that no appreciable loss of bromate occurs: (I) during mixing even for 8 minutes; (II) during a reaction time up to 3 hours; (III) during subsequent treatment by rounding in the extensograph; or (IV) during the following rest period up to 1.5 hours. Experiment V shows that no loss of bromate occurs if the final treatment is made more severe by remixing the dough for 3 minutes instead of rounding it.

As the foregoing results seemed surprising, a further set of experiments was carried out with flour B which was milled to give first patent, second patent, and clear flour, and with a different straight grade flour C. Reducing values were obtained on these flours by

TABLE II

RECOVERY OF BROMATE FROM NON-YEASTED DOUGHS CONTAINING 30 P.P.M. BROMATE

Exp. No.	Mix	Reaction Time	Treatment	Rest Period	Recovery
	min.	*hr.*		*hr.*	*p.p.m.*
I	1	..	..	..	30
	2	..	..	..	31
	3	..	..	..	32
	4	..	..	..	32
	8	..	..	..	31
II	4	1	..	..	31
	4	2	..	..	32
	4	3	..	..	29
III	4	1	round 20×	..	30
	4	2	round 20×	..	31
	4	3	round 20×	..	30
IV	4	1	round 20×	1	31
	4	1.5	round 20×	1.5	30
V	4	1	remix 3 min.	..	31
	4	2	remix 3 min.	..	30

the Chapman and McFarlane method (7). Doughs were prepared with 30 and 60 p.p.m. of bromate and one set of extensograms was obtained at zero reaction time and a rest period of 45 minutes, and another set on doughs which were allowed a reaction time of 135 minutes, followed by a 45 minute rest period before testing. Corresponding doughs allowed identical reaction times, i.e., zero and 135 minutes, were analyzed for bromate content. The results are summarized in Table III.

TABLE III

ANALYTICAL AND CORRESPONDING EXTENSOGRAPH DATA ON BROMATED DOUGHS

Flour	Reducing Value Mol. $K_4Fe(CN)_6/g. \times 10^{-5}$	30 p.p.m. bromate			60 p.p.m. bromate		
		Bromate loss p.p.m.	Ext. Ht. increase, B.U.		Bromate loss p.p.m.	Ext. Ht. increase, B.U.	
			max.	11 cm.		max.	11 cm.
B							
1st pat.	2.9	0.5	145	120	0.0	365	375
2nd pat.	3.0	3.5	190	135	1.0	520	495
clear	4.6	7.0	130	110	6.5	485	475
C							
straight	2.7	7.0	170	130	7.0	410	385

The results show that clear flour had a higher reducing value but that the other flours were not well differentiated by the reduction test. Bromate loss, however, showed a definite gradation. First patent flour

showed a negligibly small loss, second patent a definite loss, and clear a substantial loss of bromate, after a reaction time of 135 minutes. The straight grade flour C showed a loss of bromate somewhat larger than might have been expected from the results on flour stream fractions, but flour C was, of course, milled from a different wheat and a direct comparison cannot necessarily be made. It is interesting to note that while the data show a clear trend of increased bromate loss with decreasing grade of flour, the loss of bromate for each flour was independent of the amount of bromate added.

Extensogram data are presented as the difference between control doughs containing no bromate, and bromated doughs given a reaction time of 135 minutes and a rest period of 45 minutes. Both the traditional extensogram height at maximum, and the height at constant dough sample deformation of 11 cm. on the kymograph paper, were used.

The data show that while analytically bromate loss at 30 and 60 p.p.m. bromate levels was essentially the same, the increment in extensogram height was about 2.5 times greater in dough treated with 60 p.p.m. as compared with doughs which were treated with 30 p.p.m. In contrast, while the analytical data show a pronounced increase in bromate loss with decreasing flour quality, extensogram data do not show any trend with flour grade. Bromate response as measured by the extensograph does not appear, therefore, to be correlated with the reduction of bromate in dough.

A third and final set of flour-water doughs deals with the influence of pH on bromate response as measured by the extensograph and by

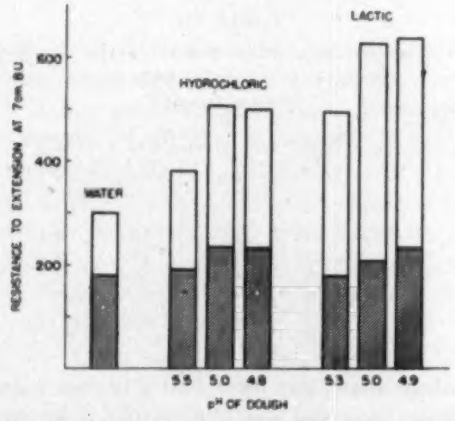

Fig. 1. Influence of acid on extensogram heights for bromate-treated doughs (total height) and untreated dough (shaded portion).

estimation of unreduced bromate. Flour D was made up into doughs containing zero and 30 p.p.m. bromate with water, and acidified with hydrochloric acid to give doughs of pH 5.5, 5.0 and 4.8 and with lactic acid to give doughs of pH 5.3, 5.0, and 4.9. Extensograms were obtained on these doughs after a reaction time of 135 minutes and a rest period of 45 minutes, and extensogram heights at 7 cm. were recorded.

The results of these experiments are shown as bar graphs in Fig. 1. The lower hatched portion of each bar represents extensogram heights for control doughs containing no bromate and made up to the pH's indicated. The total height of each bar represents extensogram heights of doughs containing 30 p.p.m. bromate at the same pH's. All doughs were tested after a reaction time of 135 minutes and a rest period of 45 minutes. The clear portion of each bar thus represents the increase in extensogram height due to the action of 30 p.p.m. bromate at the various pH's indicated.

Figure 1 shows that both hydrochloric and lactic acids slightly increase extensogram height in doughs which contain no bromate. This is indicated by the increase in the height of the hatched portions of graphs for acidified flour-water doughs. The graph also shows a very pronounced influence of acid on the increase in extensogram height of acidified doughs. Thus the increase due to bromate in flour-water dough is about 100 Brabender units, but the increase due to the same amount of bromate in dough acidified with lactic acid at pH 5.1 is more than 400 Brabender units.

Corresponding to the above extensograph experiments some analytical results were also obtained. Only lactic acid acidified doughs at pH 4.9 and 5.1, and control flour-water doughs initially at pH 5.9 were analyzed. In order to accentuate the loss of bromate a 4-hour reaction time was selected instead of 2.25 hours employed with the extensograph. As bromate reduction appeared to be small even at the end of 4 hours, reaction time of 12 hours was permitted in one experiment. The results are summarized in Table IV.

TABLE IV

BROMATE RECOVERY IN ACIDIFIED DOUGHS CONTAINING 30 P.P.M. BROMATE

Reaction Time	Recovery, p.p.m.		
	Flour water dough	Acidified Dough	
		pH 5.1	pH 4.9
hrs.			
4	25	24	25
12	18	..	15

The analytical data show no difference in the amount of bromate reduction between unacidified flour-water doughs and doughs acidified to pH 5.1 and 4.9, after 4 hours. After 12 hours reaction, however, there was 3 p.p.m. more bromate reduced in dough of pH 4.9. On this basis reduction in 2.25 hours employed in extensograph tests amounts to 0.66 p.p.m. which may be attributed to the presence of acid.

The remaining experiments deal with the decomposition of bromate in yeasted doughs, under a variety of conditions.

Table V summarizes data on the influence of treatment corresponding to various stages of test baking procedure on the recovery of bromate. Doughs were made from flour A according to the usual formula and contained 3% yeast and two levels of bromate, 10 and 30 p.p.m. A definite reduction of bromate in yeasted doughs within 4 hours is shown by the results, in general agreement with the findings of Sullivan, *et al.* (12). An initial reduction is shown on mixing, and a subsequent reduction on 4-hour fermentation. The reduction of bromate in fermenting dough containing 10 p.p.m. is only slightly less at longer fermentation times than in dough containing 30 p.p.m.

TABLE V

LOSS OF BROMATE IN FERMENTATION OF BREAD DOUGH

Original bromate level p.p.m.	Loss, p.p.m., at time, min.			
	3	45	180	235
	Mix	First punch	Mould	Full proof
10	2.5	4.5	5.0	6.5
30	2.5	4.0	8.0	9.0

Comparison of these results on yeasted doughs with the results of the first experiment on non-yeasted doughs leads to the inference that yeast in some way plays a part in the decomposition of bromate. Accordingly, a further investigation was made involving rate studies for periods up to 20 hours to obtain more information on the role of yeast. Yeast levels of 0, 1 and 3% and bromate levels of 10 and 30 p.p.m. were selected. Dough formulas were the same as for the baking test but sugar was omitted.

The results are summarized in Fig. 2. Experiments in the upper left hand corner were carried out on flour A and all the remaining experiments on flour E. The following features should be noted: first, the rate of decomposition of bromate in unyeasted doughs as shown in graphs on the left is relatively slow indicating that only small amounts of bromate decomposition would be expected during

short reaction times in dough. The rate of decomposition of bromate in unyeasted dough from flour A (top left) was slower than in unyeasted dough from flour E. Second, the presence of yeast in dough definitely increases the rate of bromate decomposition. Thus at 30 p.p.m. containing no yeast bromate decomposed at the rate of 1.2 p.p.m. per hour for flour E; from doughs containing 1% yeast bromate decomposed at 1.8 p.p.m.; and from doughs containing 3% yeast at 2.1 p.p.m. per hour. Third, the rate of bromate decomposition in yeasted doughs seems to be greater at the higher bromate level. And fourth, the rate of bromate decomposition appears to be linear with time.

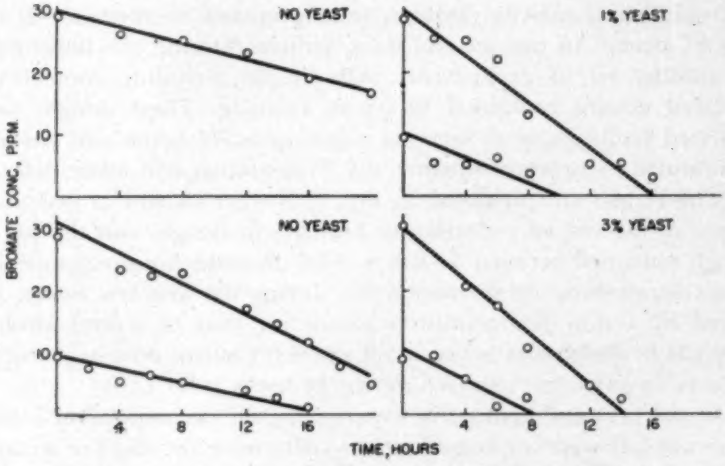

Fig. 2. Rate of bromate decomposition in doughs containing 0, 1 and 3% yeast. Upper left hand graph, flour A; all other graphs, flour E.

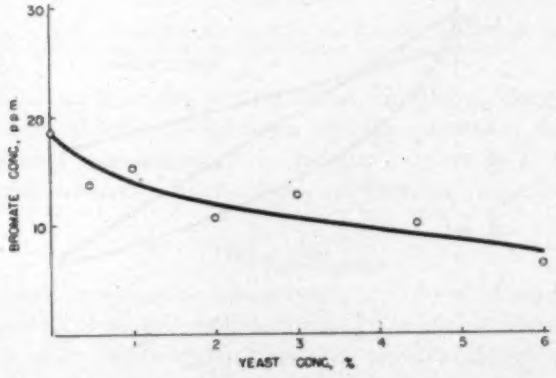

Fig. 3. Dependence of bromate decomposition on yeast content of dough.

The dependence of bromate reduction on yeast is further shown in Fig. 3. Bromate recovery was determined after doughs were fermented for 12 hours, with 30 p.p.m. bromate and concentrations of 0-6% yeast.

So far, the evidence indicates that the reduction of bromate in fermenting dough is closely connected with yeast activity. However, the results also show appreciable bromate reduction in flour-water doughs over a prolonged period. Several experiments were therefore designed to test whether this bromate loss in flour-water doughs might in part be attributed to microbiological activity of the flora present in flour.

N-octyl alcohol, and sodium fluoride were therefore used to inhibit microbiological activity. Doughs were prepared to contain 0.6 ml. n-octyl alcohol in one set and 0.3 g. sodium fluoride per 100 g. flour in another set of experiments. All doughs including control unacidified doughs contained 30 p.p.m. bromate. These doughs were analyzed for bromate at reaction times up to 24 hours and pH was determined to ascertain whether any fermentation had taken place.

The results are presented in Fig. 4. N-octyl alcohol caused a decrease in the rate of reduction of bromate in dough, and the pH of dough remained between 5.8-6.0. Sodium fluoride, however, appeared to accelerate bromate decomposition during the first few hours, followed by a slow decomposition paralleling that of n-octyl alcohol. The pH of dough was between 5.9 and 6.1. Control doughs showed a decrease in pH from 5.9 to 5.6 during 24 hours at 30°C.

In the next and final set of experiments the decomposition of bromate was followed in sponge doughs to determine the effect of accumulated metabolic products of yeast. In these doughs yeast fermentation

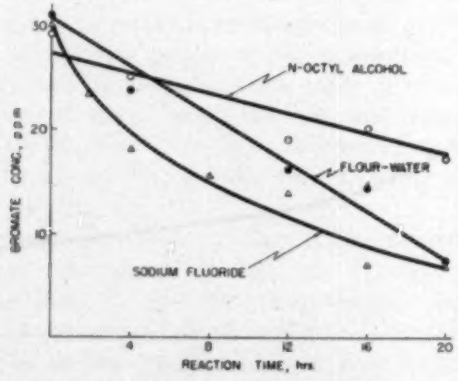

Fig. 4. Influence of yeast inhibitors on the rate of bromate decomposition in flour-water doughs.

was allowed to proceed for 3 hours and for 12 hours in the sponge which contained 70% flour and water and all of the dough formula except bromate. At the end of this time the sponge and the remainder of the water containing 30 p.p.m. bromate was mixed for 1¼ minutes to a gruel-like consistency, then the remainder of the flour was added and the dough mixed for a further ¾ minute. Doughs were analyzed for bromate at 0 to 15 hours after doughing up. While this work was in progress the Howe method for bromate determination (8) was published. This set of experiments on sponge doughs was therefore analyzed by both methods. The data are summarized in Fig. 5. There is little to choose between the precision of the Johnson and Alcock and the Howe method for bromate analysis in dough. The results by the Howe method tend to give slightly higher results at long fermentations.

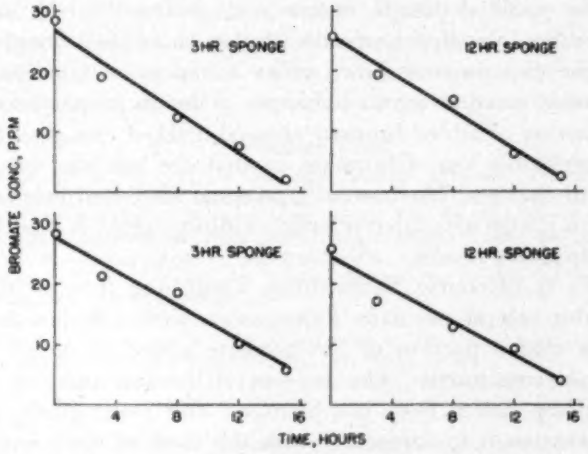

Fig. 5. Rate of bromate decomposition in sponge doughs—above by Johnson and Alcock method, below by Howe method.

The results on bromate decomposition in sponge doughs are on the whole very similar to the data already presented for straight doughs. There is no indication that metabolic products of yeast have an appreciable influence on the course of bromate reduction.

Discussion

The present investigation was initially conceived to study to what extent the action of potassium bromate as an oxidizing agent is related to its action as an improver in modifying physical dough properties. Let us examine the evidence obtained.

Experiments on flour-water doughs present parallel data on the oxidizing action of bromate measured as bromate decomposition, and on changes in physical dough properties resulting from the action of bromate in dough measured as changes in extensograms. In one set of experiments no significant loss of bromate could be shown analytically under conditions which are known to produce marked changes in extensogram dimensions. In another set of experiments analytical data showed a definite trend of bromate loss in the direction of flour of lower quality; extensogram data on identical doughs showed no such trend. Moreover, while analytical data showed no significant difference between bromate loss at 30 and 60 p.p.m. levels for each flour, the increase in extensogram height in parallel doughs was about 2.5 times as large for the higher bromate level. Finally the influence of acid, down to pH 4.9 was to increase the response to bromate markedly, judged by the increase in extensogram height, but the loss of bromate in acidified doughs determined analytically was only very slightly greater in comparison with similar unacidified doughs.

All these experiments show, under a variety of conditions, that while physical measurements of changes in dough properties resulting from the action of added bromate showed marked changes, there was either a negligible loss of bromate, or that the loss was unrelated to the physical changes. The currently accepted view that bromate as an improver in dough acts directly as an oxidizing agent is inadequate to explain the above results.

Bromate is, of course, an oxidizing agent, and it is of interest to examine this role of bromate. Experiments with different flour fractions show that a portion of the bromate added to dough oxidizes certain flour constituents. The amount of bromate used in this way is small in top patent flour but increases with lower grades of flour. This observation is in agreement with the work of Shen and Geddes (10) and others who found that the reducing content increases with decreasing grade of flour, and with the general mill experience that lower grades of flour require a higher bromate treatment. It is quite reasonable to suppose that oxidation of certain deleterious substances may be a sort of negative improver action in neutralizing their harmful action. But positive and progressive improver effect of bromate as shown by an increased resistance to extension when doughs are tested on the extensograph appears to be unrelated to this part of the oxidizing action of bromate.

Work with yeasted doughs shows that yeast in some way plays a definite part in the decomposition of bromate. There is a definite bromate loss in pre-oven stages of bread dough and a very large loss

during longer periods. Judging from results on sponge doughs it appears that there is no significant accumulation of reducing metabolic products in the dough as a result of yeast fermentation. In other words the activity of the yeast cell itself seems to be involved in the reduction of bromate. Evidence has also been obtained to suggest that microbiological action in flour-water doughs is responsible for some loss of bromate during prolonged periods. It is important to remember, however, that yeast and other flora are extraneous to flour, and the loss of bromate by this mechanism cannot therefore be associated with improver action in the dough.

The loss of bromate in dough as a measure of its oxidizing action may thus be attributed to at least three factors, which in order of decreasing magnitude are, yeast activity, reducing substances in flour, and indigenous microbiological activity of flour. So far it has not been possible to associate any portion of the oxidizing action of bromate in flour with its improver action in dough. Perhaps a more precise method for bromate determination in dough (not flour) might yield additional information. It is not unlikely, however, that the solution for the mechanism of bromate improver action lies elsewhere.

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STUDY OF PROTEOLYTIC ACTIVITY IN WHEAT FLOUR DOUGHS AND SUSPENSIONS. III. THE MISCLASSIFICATION OF THE PROTEASES OF FLOUR AS PAPAINASES[1]

B. D. HITES,[2] R. M. SANDSTEDT, and LORENE SCHAUMBURG

ABSTRACT

The proteolytic enzymes of wheat flour are not papainases since they are not activated by reducing agents nor inactivated by oxidizing agents. Proteolysis great enough to produce notable physical changes in doughs may be produced by added "foreign" enzymes and this action may be detected and determined by chemical procedures without the use of a supplemental susceptible substrate. However, only slight proteolysis can be detected in doughs in the absence of "foreign" enzymes without the use of a "foreign" substrate; in other words, the enzymes of flour have practically no action on the proteins of flour.

This study of the proteolytic enzymes of wheat flour was initiated as a result of the continued disagreement concerning the mode of action of oxidizing and reducing agents in doughs. The explanation proposed by Jørgensen (9) and Balls and Hale (2, 3, 4) that the observed effects of oxidation and reduction are due to inhibition and activation of the proteolytic enzymes of flour, is essentially based on the classification of these proteolytic enzymes as papainases (3). Reed and Haas (13) presented evidence indicating that these enzymes were not papainases. However, their designation as papainases has been generally accepted and not seriously questioned even by those who did not subscribe to the Jørgensen-Balls and Hale theory. Objection to this theory was based on the question of whether there was enough enzyme, even though activated, in flour to be of consequence in baking and also whether the reducing and oxidizing agents have a direct action on gluten. The literature on these questions was adequately reviewed by Hildebrand (6).

Samuel (14) reported that extracts of flour showed no increase in amino nitrogen on autolysis—in other words, the soluble enzymes had no action on the soluble proteins. However, it has been assumed that "appreciable peptide scission in water extracts of flour is to be expected since they certainly contain both enzyme and substrate, and the substrate is in a form rather easily attacked by the enzyme"

[1] Manuscript received January 26, 1953. Presented at the annual meeting American Association of Cereal Chemists, Chicago, May, 1950.

[2] Present address, Department of Agriculture and Inspection, Nebraska State Capitol, Lincoln, Nebraska.

Published with the approval of the Director as Paper No. 604, Journal Series, Nebraska Agricultural Experiment Station.

(8). Olcott, Sapirstein, and Blish (12) showed that the insoluble enzymes apparently have a slight action on the gluten proteins. Sandstedt and Fortmann (15) showed that the slackness and stickiness imparted to doughs by reducing agents could be reversed, even after a 4-hour fermentation period, by the subsequent addition of an oxidizing agent and that these doughs then made normal bread. On the other hand, poor baking properties imparted to doughs by papain action could not be eliminated by subsequent oxidation. This difference in reversibility indicates that the softening action of the reducing agents on the doughs was not due to proteolysis.

Since the purpose of this paper is to show similarities and differences between the flour enzymes and papainases, their action both on hemoglobin and on flour protein and their activation and inactivation are directly compared using papain as a model or "standard" papainase.

Materials and Methods

Flour. Three commercially milled unbleached hard winter wheat flours were used: an 85% patent containing 11.3% protein and 0.43% ash, a first clear with 12.9% protein and 0.61% ash, and a second clear with 15.1% protein and 0.86% ash (14% moisture basis).

The hemoglobin was Difco Bacto-hemoglobin.

The papain was a standardized sample obtained from Merck and Company.

Proteolysis. The Ayre-Anderson method (1) for the determination of proteolysis with its modifications (10, 11) was used in this study. The method as used involved the autolytic digestion at 40° C. of 10 g. of flour with 50 ml. of solution containing 2.5 ml. of 4 *M* acetate buffer, pH 4.7.[3] In case hemoglobin was used as a supplemental substrate in addition to the flour, 1.25 g. was mixed with the 10 g. of flour before adding the liquids, or in case the hemoglobin was the entire substrate, the 1.25 g. of hemoglobin replaced the 10 g. of flour.

At the end of the digestion period the suspension was clarified by thoroughly mixing with 4 ml. of 45% sodium tungstate followed by 12 ml. of 5 *M* trichloracetic acid.[4]

The precipitation was followed by centrifuging at 11,000 rpm.

[3] The acetate buffer was satisfactorily effective as a preservative. The use of toluol, octyl alcohol, dehydroacetic acid, and Roccal should be avoided as they have marked effects on proteolysis under certain conditions.

[4] It was not always possible to obtain clear filtrates by clarification with trichloracetic acid alone. Cloudy filtrates indicate incomplete precipitation which may cause discrepancies due to variability in rates of adding the precpitating agent, rates of stirring, centrifugal speeds, and filtering techniques. Water clear filtrates were obtained with the sodium tungstate-trichloracetic acid procedure.

(15,000×G) for 5 minutes with subsequent decantation through a No. 4 Whatman filter paper. Aliquots of from 25 ml. to 40 ml., depending on the nitrogen present, were taken for Kjeldahl determinations. The nitrogen found was calculated to total non-precipitable nitrogen (NPN) per 10 g. of flour and this figure was then corrected to represent the NPN produced by enzyme action on 10 g. of flour. Suitable control tests were made for all periods of time covered by the particular experiment and the data presented have been corrected by subtraction to give the figures of direct interest.

Results and Discussion

Effect of Sulfhydryl Compounds. The data in Fig. 1 confirm pre-

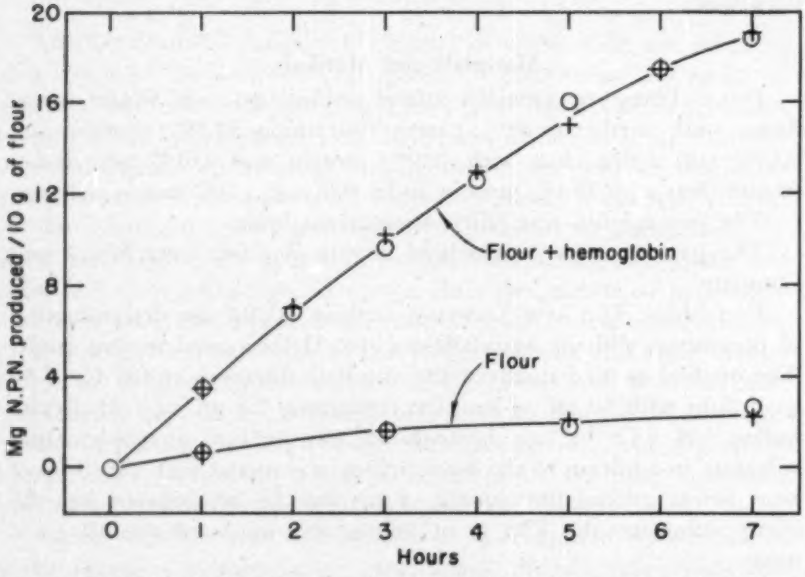

Fig. 1. The action of native flour enzymes on the proteins of flour and on flour + hemoglobin. Ten grams of unbleached 1st clear flour or 10 g. of flour + 1.25 g. of hemoglobin in 50 ml. of pH 4.7 acetate buffer digested 4 hours at 40° C. O — indicate data obtained without added reducing agents. + — indicate data obtained with 1.5 mg. (flour basis 15 mg. %) of added glutathione.

vious work showing that though the proteases of flour have little action on the flour proteins, they are active on some other proteins such as hemoglobin (10, 11) casein (5, 8) and gelatin (13). These have been used as susceptible substrates to demonstrate the presence of active proteases in flour.

By definition, the papainases are proteolytic enzymes which are activated by certain reducing agents, and are inactivated by certain oxidizing agents. Figure 1 shows that the presence of 1.5 mg. (15 mg.% on a flour basis) of glutathione (GSH) had no stimulating effect on the flour proteases acting on either the flour proteins or on flour plus hemoglobin. However, that 10, 15, and 100 mg.% of GSH cause

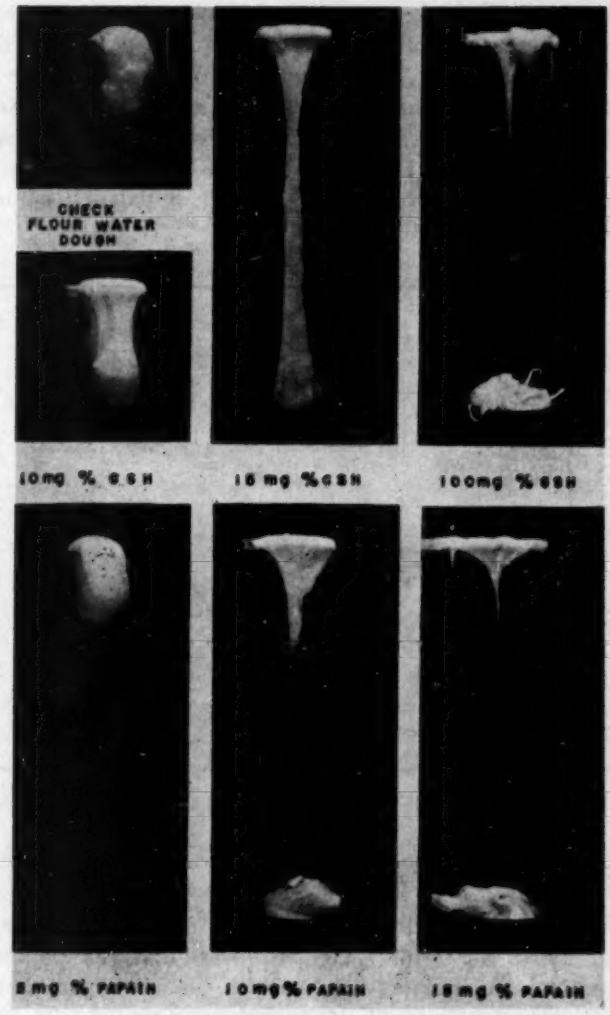

Fig. 2. A comparison of liquefaction of doughs produced by glutathione and by papain (proteolysis). Four-hour flour-water doughs suspended on the glass rod 2 minutes before photographing.

increasingly slack, "runny" doughs, is illustrated by the photographs of Fig. 2, but the liquefaction was accompanied by no measurable proteolysis (Fig. 1 and Table I).

It has been claimed that such slackening without measurable proteolysis is due to early stages of proteolysis, i.e., to cleavage into large peptides which would cause marked changes in the physical character of a dough but which would not be detectable by chemical methods (4). If this were true it should be possible to produce an equivalent liquefaction of dough by means of papain with no determinable non-precipitable nitrogen (NPN) production. The slackening of doughs by the addition of varying quantities of papain also is shown in Fig. 2. The effect of 5 mg.% was slight—corresponding to the chemical data of Fig. 3. As previously shown, the lack of action with less than approximately 6 mg.% of papain is due to the presence of a papain inhibitor (7).

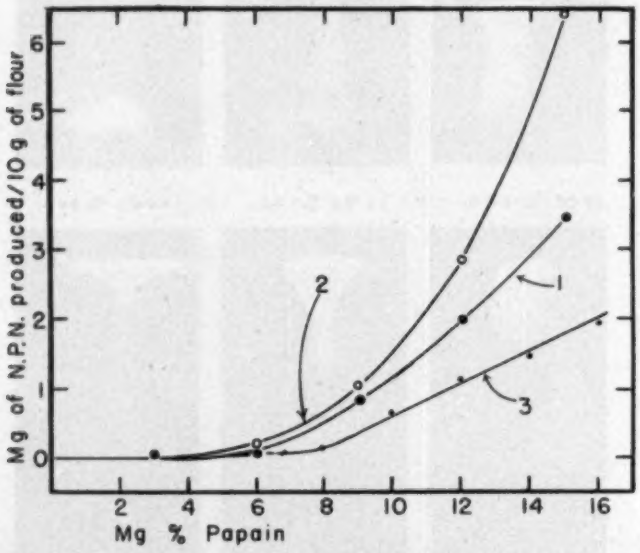

Fig. 3. Action of papain on unbleached bakers patent flour. Ten grams of flour digested 4 hours. Action of increasing increments of papain: 1, on flour; 2, on flour with 1.5 mg. of GSH; 3, on flour with 0.3 mg. of sodium chlorite.

The liquefaction with 10 mg.% of papain was similar to that produced by 15 mg.% of GSH. However, that the papain action which produces such slackening may be chemically measured is indicated in Fig. 3: 15 mg.% of papain produced 3.5 mg. of NPN per 10 g. of flour during a 4 hour digestion; this represents only

about 2% of the total nitrogen of the flour, but this quantity, though small, is readily determinable. Accordingly, actual proteolysis great enough to produce notable slackness should be chemically determinable and conversely, marked changes in the physical characteristics of doughs that are not detectable by adequate chemical methods probably do not involve proteolysis.

Since the flour proteases did not respond to 15 mg.% of GSH, it should be noted that further increases in the concentration of sulfhydryl did cause drastic liquefaction (Fig. 2) which, however, was not accompanied by an increase in NPN. Table I shows the data obtained by the use of 150 mg.% of cysteine. GSH gives the same type of negative results.

TABLE I

THE EFFECT OF CYSTEINE ON THE ACTION OF THE NATIVE PROTEASES OF FLOUR[1]

Time of Digestion	NPN Produced	
	Flour without Cysteine	Flour + 150 mg.% Cysteine
hrs.	*mg/10 g.*	*mg/10 g.*
1	1.4	1.5
2	2.4	2.4
4	3.9	3.8
6	4.9	4.7

[1] Second clear flour suspensions autolyzed at pH 4.7 and 40° C. The NPN data obtained from 15 minute digestions were substracted as "blanks".

That a true papainase is activated by GSH under conditions similar to those of Fig. 1 is indicated by a comparison of curves 1 and 2 of Fig. 3 which show the action of increasing quantities of papain on a first clear flour without (curve 1) and with (curve 2) 15 mg.% of GSH. Figure 4 compares the action of 1.5 mg. of papain on 1.25 g. of hemoglobin without (A) and with (B) 1.5 mg. of GSH. In these cases there is no question of the activation. These data indicate the amount of activation that should be expected from enzymes of the papainase type, whether acting on flour proteins or on hemoglobin.

Effect of Oxidizing Agents. Since the flour proteases did not respond to the presence of GSH, would they respond to the presence of the oxidizing agents which effectively inhibit the papainases? Figure 5 illustrates the variation in NPN observed with flours of differing grades and shows the lack of proteolytic inhibition obtained using sodium chlorite as an oxidizing agent on flour suspensions and on flour-hemoglobin suspensions. The enzymes were not inhibited by the

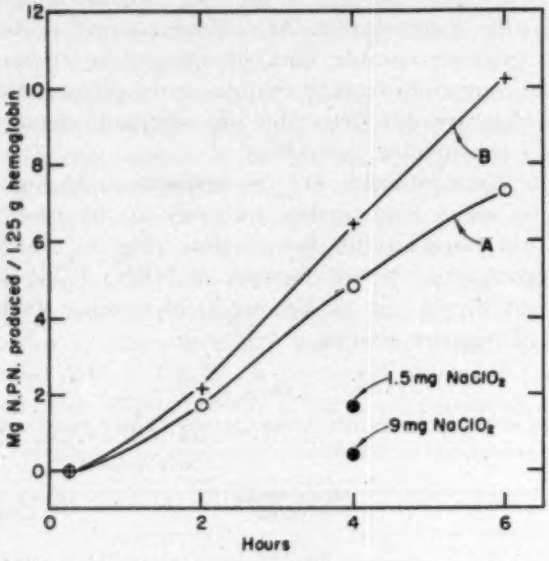

Fig. 4. Action of papain on hemoglobin; 1.25 g. of hemoglobin digested with 1.5 mg. of papain: A, no added reducing or oxidizing agents; B, with 1.5 mg. of glutathione; ●, with indicated quantity of sodium chlorite.

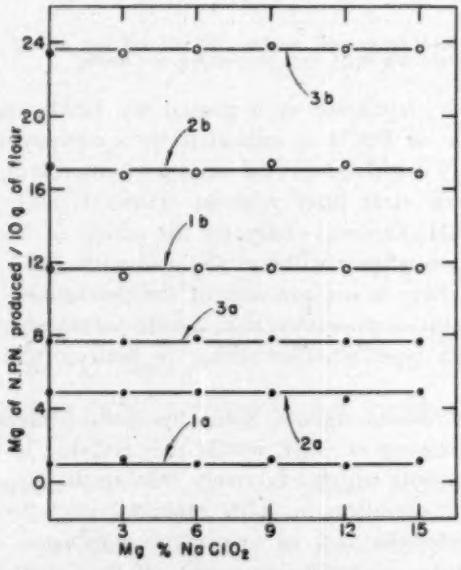

Fig. 5. The ineffectiveness of sodium chlorite for inhibiting the native flour proteases: Curves 1a, 2a and 3a represent data obtained from 4 hour digestions of 10 g. of unbleached patent, 1st clear and 2nd clear flours; Curves 1b, 2b, and 3b from digestions of the flours plus 1.25 g. of hemoglobin.

presence of 15 mg.% of sodium chlorite (on a flour basis). The same lack of inhibition was obtained when the chlorite was increased to 300 mg. %. Negative results also were obtained with bromate and iodate. However the action of a papainase was effectively inhibited under these conditions (Fig. 6). Papain in suspension with hemoglobin seems to be more difficult to inhibit than papain in suspension

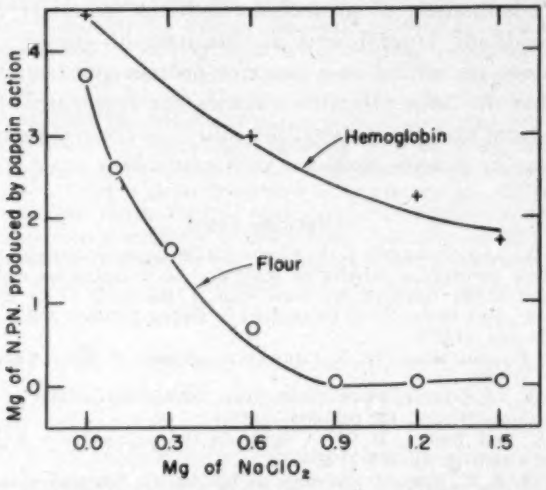

Fig. 6. Inhibition of papain action by chlorite: 10 g. of unbleached patent flour digested 4 hours with 1.5 mg. of papain and increasing increments of sodium chlorite; also 1.25 g. of hemoglobin as above with 1.5 mg. of papain and increasing quantities of chlorite.

in flour. These results are substantiated by data included in Figs. 3 and 4.

Discussion

The studies show that reducing agents did not activate the native proteases of wheat flour. Conversely, oxidizing agents did not inactivate these enzymes. Accordingly it is concluded that the native flour proteases are not papainases.

Papain action great enough to produce notable physical changes in doughs may be detected and determined by chemical procedures without the use of susceptible supplemental substrates. The use of supplemental substrates to illustrate the enzyme action in doughs may be misleading and results of such studies should be interpreted with caution. Physical changes in doughs (consistency, extensibility, plasticity, viscosity, fluidity, stickiness, or other handling properties) may or may not be due to proteolysis and accordingly are not reliable criteria of proteolysis.

Since the present studies indicate that the flour proteases are not papainases, the general acceptance of such misclassification requires further explanation especially since this acceptance was based on analytical data obtained not only by physical methods but also by chemical methods. The apparent activations and inactivations which have been reported for the flour proteases have been small when compared to activation and inactivation of papain.

The determination of proteolysis with the necessary controls becomes exceedingly complicated as mixtures of enzymes, inhibitors and activators are added to a reactive protein substrate. It is to be expected that the large protein molecules (or aggregates of molecules) of the substrate may react sluggishly and that the results of such slow reaction may be readily confused with proteolysis.

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A METHOD OF DETERMINING ENZYMATIC DIGESTION OF RAW STARCH[1]

ROBERT L. GATES[2] and R. M. SANDSTEDT

ABSTRACT

A gravimetric method for determining *in vitro* digestion of raw starch is presented. Essentially the method consists of digesting 0.5 g. of starch for 20 hours with constant stirring in 20 ml. of solution containing the necessary buffer, microorganism inhibitor and enzyme. The undigested residue is filtered, dried and weighed. The loss in weight is the measure of enzyme activity. Additional information is obtained by determining the reducing value of the solubilized degradation products. Data are given showing the relation of enzyme concentration and time of digestion to the quantity of starch digested. Using a given bacterial enzyme preparation, the amount of raw starch digested which could be accounted for as maltose (reducing value determination) was a relatively constant factor (64–73%) for all conditions studied. A raw starch digestion unit is proposed, namely, the enzyme activity required to accomplish 20% digestion of 1 mg. of raw corn starch in 20 hours.

The ability of some amylases to digest raw starch, an ability which is frequently not appreciated, may be demonstrated by using a high concentration of enzyme and permitting a long digestion period. Reichert (7) gives an extensive review of studies showing the action and lack of action of malt and of salivary enzymes on raw starches. The wide variations in digestibility of the starches from various species of plants are reported. These early studies were largely based on microscopic observation. More recently the digestion of raw starch has been followed by the determination of reducing substances produced (8, 1, 6, 10). However, there is difficulty in the interpretation of reducing values since the products of raw starch digestion have not been adequately defined. This difficulty is particularly apparent when different enzyme sources and mixtures of enzymes are being compared. The problem of interpreting reducing value data also applies to digestions of boiled starch.

The method presented in this paper for the assay of enzymic activity on raw starch utilizes the simplest possible approach. Uncooked starch has the convenient property of undetectable solubility in water at temperatures below 40°C. By using the simple expedient of recovering the insoluble residue after a period of digestion of a known

[1] Manuscript received January 26, 1953. Published with the approval of the Director as Paper No. 581 Journal Series, Nebraska Agricultural Experiment Station.

[2] Present address: Westvaco Chemical Division, Food Machinery and Chemical Corporation, South Charleston 3, West Virginia.

quantity of starch an indication of the amount of enzymic activity can be obtained. The method does not require an arbitrary interpretation of the kind of soluble reducing materials that are produced during the course of digestion.

Materials and Methods

Enzyme Source. The enzyme preparation was a special concentrate of a commercial bacterial amylase preparation.[3] It had an alpha-amylase activity of 6,100 SKB (9) units when buffered at pH 6.5 with M/15 phosphate.

Starch Substrate. The raw starch substrates were samples of commercial corn, wheat and potato starches. The wheat starch used in the preliminary study was a commercial preparation from which the small granules were discarded by differential sedimentation.

Buffer. M/7.5 phosphate — pH 6.5: 1.14 g. dibasic sodium phosphate heptahydrate + 1.23 g. monobasic potassium phosphate per 100 ml.

Microorganism Inhibitor. The 20 hour period suggested for the digestion of raw starch necessitates precautions for preventing microorganism contamination and growth. Chemicals used for the prevention of microorganism growth should not affect the action of the enzyme under study. For this study "Roccal" (a detergent sold by Sterwin Chemicals, Inc.) was used at a 0.1% concentration. However, Roccal stimulates raw starch digestion under these conditions (4) and accordingly is not recommended. Chloroform (0.5%) or DHA-S[4] (0.1%) have been found satisfactory for raw starch digestion.

Reducing Value. The determination of reducing value was by the ferricyanide method as outlined by Kneen and Sandstedt (5).

Raw Starch Digestion. To 0.5 g. of raw starch in a 25 ml. volumetric flask were added 10 ml. of buffer solution, an aliquot of the enzyme solution, the microorganism inhibitor, and water to bring the volume to 20 ml. The flask was placed in a wrist-type shaker with the bulb of the flask immersed in a 30°C. water bath. The amplitude of shaking was adjusted to the minimum necessary to maintain the starch granules in suspension. After 20 hours digestion, the mixture was made to 25 ml. volume with 1.0% sulfuric acid solution (which gives an approximate pH of 2 and inactivates the enzyme) and filtered through a tared fritted Pyrex glass crucible of fine porosity (maximum pore size 5.5 microns). The residual starch was washed with three 20 ml. portions of distilled water followed by two ml. portions of alcohol. (If the filtrate is to be used for further analysis, it should be removed

[3] Concentrate of "Rhozyme DX" furnished by Rohm and Haas Company, Philadelphia, Pa.
[4] The sodium salt of dehydroacetic acid sold by the Dow Chemical Company.

before the washing.) After drying to constant weight in a vacuum oven at 100°C., the loss in weight of the starch is the index of enzyme activity.

Routinely, in this laboratory, 7 g. of the raw starch substrate is suspended in 65 ml. of water in a 100 ml. Erlenmeyer. The starch is kept in suspension with a magnetic stirrer while 5 ml. aliquots are transferred to 25 ml. volumetric flasks. (The starch recovered from a series of 5 of such aliquots ranged from 478.9 mg. to 481.0 mg., giving a maximum deviation of 1.2 mg. or 0.25% from the average.) The buffer, microorganism inhibitor and enzyme are added. The digestion and determination of residual starch are carried out as described above. Ten flasks are included in each run with no enzyme added to the first and last. The starch recovered from these blanks is used as the basis for calculations, i. e., as the amount of insoluble starch originally added to the flasks.

Results and Discussion

Excellent day to day replication of results is obtained by this method. For example, in a series of eight determinations in which the pH was varied from 5.8 to 7.2, the maximum variation between the duplicates (run on different days) was 5.0%; the average variation was 2.9%.

As a check on the weight loss method of determining the amount of starch digested, the filtrate from each digest was analyzed for reducing value by ferricyanide reduction (5). The amount of ferricyanide reduced was arbitrarily calculated as milligrams of maltose. This in turn was calculated to the amount of starch (maltose $\times$ 0.95) hydrolyzed. Table I presents a comparison of the weight loss and reducing value methods using varying concentrations of the enzyme system. With this particular enzyme only 68% $\pm$ 5% of the starch solubilized could be accounted for as maltose produced. This ratio, which indicates incomplete conversion of starch to reducing sugars, is characteristic of this enzyme preparation. The slightly increasing value for the ratio with increasing enzyme may indicate a slow digestion of residual dextrins. The relatively constant ratio between the reducing value and weight loss data shows good agreement between methods.

Relation Between Enzyme Concentration and Extent of Raw Starch Digestion. Using an M/15 phosphate buffer at pH 6.5 and a Roccal concentration of 0.10%, a series of determinations was conducted to establish the relation between the relative amount of enzyme present and the extent of starch digestion. The range of con-

centrations studied varied from 4.1 to 62 mg. of enzyme per gram of corn starch. Figure I presents the results of these determinations. It is obvious that the first increment of enzyme added was the most

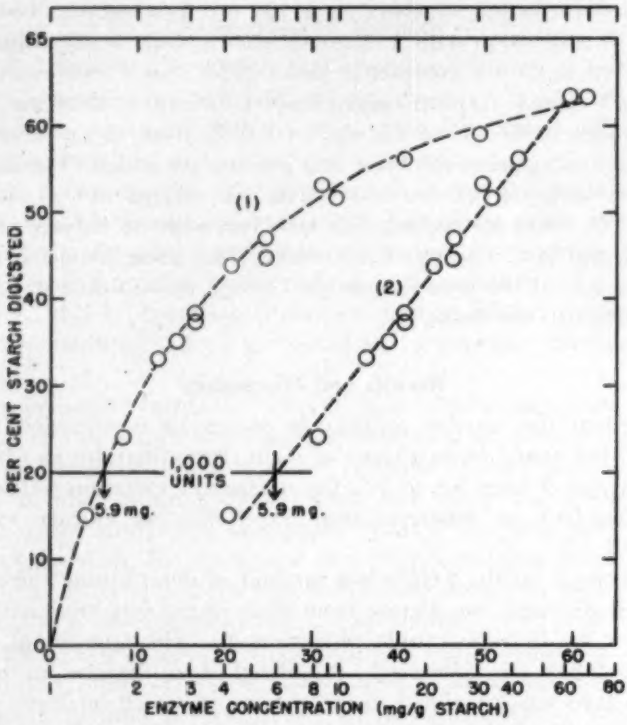

Fig. 1. Relation between enzyme concentration and the extent of raw corn starch digested by a bacterial amylase. Curve 2—Enzyme action plotted against the logarithm of concentration.

effective with a progressive decrease in effectiveness with increasing concentration. According to Curve 2 (concentration on a logarithmic scale) the extent of digestion was proportional to the log of the enzyme concentration.

Effect of Length of Time of Digestion. The influence of the time of digestion on the amount of starch digested by a constant amount of enzyme is shown in Fig. 2. The effectiveness of the enzyme decreased progressively with time. There is a similarity between the results of this study and those obtained by varying the enzyme concentration. The first hour of digestion gave more than twice the amount of digestion realized in the five successive one hour intervals. However plotting time on a logarithmic scale does not give a straight line curve.

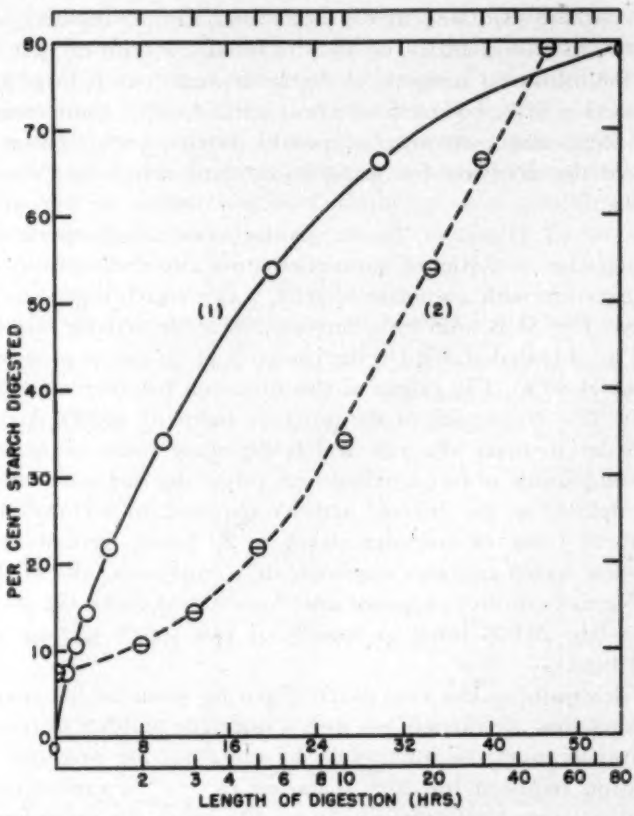

Fig. 2. Relation between the length of time of digestion and the extent of raw corn starch digestion. Curve 2—Enzyme action vs. logarithm of time of digestion.

These curves may be interpreted as indicating that the first starch attacked is the most susceptible and that the residue is increasingly resistant as the more susceptible starch is removed. However, other factors such as enzyme inactivation due to the presence of the end-products of digestion (10) complicate the picture.

Influence of Type of Starch. Reichert (7) reviews the extensive early work showing the wide variability in the digestibility of the native starches from different species of plants. Recent studies (2, 11) confirm these early observations of the resistance of some root starches as compared to the cereal starches. Accordingly it was desirable to ascertain the variation which could be expected using different starches with the weight loss method. The wheat starch which had been used in the preliminary work was a special sample of large granule wheat starch prepared by differential sedimentation. The com-

mercial starches available in the laboratory were compared with it. Using a constant quantity of the bacterial concentrate (29 mg./g. starch) the following amounts of digestion were found: large granule wheat starch – 52%, commercial wheat starch – 37%, commercial corn starch – 53%, and a commercial potato starch – 4.6%. These values emphasize the necessity for using a standard starch for raw starch digestions.

Raw Starch Digestion Units. To facilitate comparisons of raw starch digestion activities of various enzymes and comparisons of raw starch digestion with α-amylase activity, a raw starch digestion unit is proposed. The SKB unit for α-amylase (9) is the activity required to digest 1 g. of boiled starch (in the presence of an excess of β-amylase) in 1 hour at 30°C. The extent of this digestion has been shown (3) to represent 30% conversion of the starch to reducing sugars. Accordingly, in order to make the raw starch digestion value of an enzyme source comparable to its α-amylase unit value, the raw starch digesting unit is defined as the enzyme activity required to accomplish 20% digestion of 1 mg. of raw corn starch in 20 hours. Actually, then, a preparation which contains approximately equal amounts of the two types of activity on this proposed unit basis would digest the β-amylase limit dextrin 20,000 times as rapidly as raw starch (g./mg. and 20 hours/1 hour).

For determining the raw starch digesting value of an enzyme in units, digestions are carried out with a sufficient number of concentrations (two or more) to interpolate by graphing the quantity of the preparation required for 20% digestion of 1 g. of raw starch (this quantity contains 1000 units). Figure 1 illustrates the procedure. The point at which the curve crosses the 20% digestion level is the index of the quantity of enzyme preparation containing 1000 units of raw starch activity—in this particular case 5.9 mg. contained 1000 units or 1 mg. had a value of 170 units. A statement of the buffer and pH used should accompany the value in units.

Discussion

The method proposed for the determination of raw starch digestion is simple, convenient and reliable. It does not require a knowledge of the reducing substances produced for interpretation.

The method may require the use of relatively pure enzyme extracts because any insoluble material which is added or which may develop during digestion, such as coagulated protein, would be interpreted as undigested starch. In general however, by the judicious use of extracts from enzyme preparations, the amount of solids repre-

sented by the enzyme aliquot is less than 2% of the amount of starch present. Under these conditions the error introduced would be within the replicability of the method even though a considerable part of the extract solids appear in the filtered starch. In applying this method to the assay of concentrated extracts of protein-containing materials, the possibility of protein coagulation or excessive adsorption on the starch should be appreciated and evaluated as a possible cause of erroneous interpretation of results.

TABLE I

COMPARISON OF WEIGHT-DIFFERENCES AND REDUCING-VALUE METHODS OF DETERMINING RAW STARCH DIGESTION

Enzyme Concentration	Raw Starch Digested		
	By weight loss (A)	By reducing value (B)[1]	B/A
mg./g. starch	%	%	
4	15.1	10.0	0.66
8	24.0	15.4	0.64
17	37.4	25.0	0.67
25	44.6	29.8	0.67
33	51.6	34.5	0.67
41	56.2	38.5	0.68
49	59.0	41.6	0.70
60	63.3	46.0	0.73

[1] Based on the reducing value of maltose.

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USE OF COBALTOUS CHLORIDE TO DETECT MOISTURE PATTERNS IN PARTIALLY DEHYDRATED KERNELS OF CORN[1]

C. B. WARD, JR.[2] AND R. G. TISCHER[3]

ABSTRACT

The internal moisture pattern in partially dehydrated kernels of corn was determined by means of a cobalt chloride-methyl alcohol solution which changes color upon contact with moisture. Moisture contents from about 70% to 0.5% were tested.

Observations on fresh sweet corn kernels dried in a vacuum oven indicated that the drying progresses in such a manner that the residual moisture is located near the center of the kernel. When the corn kernels cut from the ear of corn were dried in hot air at atmospheric pressure, the moisture in the kernel apparently diffused to the cut surface, the residual moisture being found at the surface of the kernel.

The limit of sensitivity of the cobalt chloride solution to moisture occurred when the corn kernels contained approximately 4% moisture.

Cobalt salts have for some time been used as indicators of humidity and moisture. Cobalt chloride when dehydrated is blue, and when gradually rehydrated changes color from blue to violet, to pink, to dark red. Solomon (5) described a method of using paper impregnated with cobalt chloride to measure humidity in small spaces, such as culture jars. Papers more suitable for use at high humidities were prepared by using cobalt thiocyanate solution.

Cobalt chloride paper has long been used by plant physiologists for measuring the "transpiring power" of leaves, a phrase used by Livingston and Shreve (4) to denote the ability of the leaf to give off water vapor. To measure transpiring rates, a standardized hygrometric paper slip is usually placed upon a plant leaf and covered with a glass plate. The time required for the paper to change from one standard color to another may be converted to an hourly transpiration rate, or a transpiration coefficient, by comparing this time with the time taken by another standard paper when in contact with a standard evaporating surface. Painted on insects, cobalt chloride in collodion has been used by Eder (3) as an indicator of cuticular transpiration. When the amount of moisture is varied, color changes occur in various salts

[1] Manuscript received December 1, 1952. Journal Paper No. J-2183, Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1052. This paper reports research sponsored by the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned number 402 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of Defense.

[2] Graduate assistant, Food Technology, Iowa State College, Ames.

[3] Professor of Animal Husbandry and Horticulture, Iowa State College, Ames.

other than those of cobalt, and these changes have been used to make approximate quantitative determinations of moisture in grains and other dry materials (2).

In the dehydration of sweet corn, the rate of drying is a function of the permeability of the corn kernel to moisture, and in general, of the conditions imposed in the drying operation. It may be surmised that the distribution of moisture in the kernel during drying will affect the rate at which drying takes place.

In the present study, an attempt has been made to formulate a method for locating the moisture in the corn kernel during progressive drying. Use was made of color changes resulting from the hydration of cobalt chloride.

Materials and Methods

The cobalt chloride solution was prepared by dissolving 10 g. of crystalline cobalt chloride ($CoCl_2 \cdot 6H_2O$) in 5 ml. of 99.5% methyl alcohol. The resulting solution was deep blue.

Fresh, hand-cut corn was used for all determinations. Corn of varying moisture content was employed without distinction other than to take this moisture difference into account in calculations. Moisture contents ranging from approximately 70% to 0.5% were tested. For blending certain corn samples an Osterizer was used. This device is similar to a Waring Blendor, but equipped for use with a screw-top glass jar to contain the sample.

The cobalt chloride–methyl alcohol solution was applied to the corn as a very fine mist by means of a hand-operated atomizer. Enough was applied to cover completely the freshly exposed corn surface. Corn kernels were sliced into various sections before the cobalt chloride spray was applied to the freshly exposed surfaces. No special account was taken of the effect of the corn germ on the distribution of moisture.

In the observation of kernel sections, the partially dehydrated section was pinned on a cork slab in the field of a low-power (20.5×), wide-field microscope. The microscope was placed in a horizontal position at eye level to facilitate observation of the kernel section during and following spraying. The location of the moist (pink) portion of the section was recorded within 10 seconds after spraying was completed, unless otherwise specified. At times, observations longer than 10 seconds were made. Observations of the distribution of moisture in kernels were accompanied, for each ear of corn used, by a vacuum-oven determination of moisture using the method of the Association of Official Agricultural Chemists (1). Very thin layers

of blended corn were also tested with the cobalt chloride spray in an effort to determine the limit of sensitivity of the test at low moisture levels.

For dehydrating the corn, three methods were used: (a) the vacuum-oven, (b) the hot-air oven, and (c) a stream of hot air. The vacuum-oven method was carried out as follows: The weighed corn sample (blended, or in kernel form) was placed in a tared aluminum dish previously dried in the vacuum oven, and was dehydrated at 95°–100° C. and 0.01 mm. mercury pressure until the moisture content of the corn was judged to be at the level desired. Upon removal from the oven, the sample was cooled in a desiccator containing calcium chloride and quickly reweighed with the dish closed. Percent moisture could then be calculated from knowledge of total weight loss.

Using the hot-air oven, the procedure was the same, except that three different temperatures were used: 90°, 110°, and 130°F. (32°, 43°, and 54° C.) for different samples.

Corn kernels were dried in a stream of hot air by placing them on a stainless-steel wire screen fixed above a hot plate so that the temperature at the level of the screen was approximately 110°F. (43°C.). Dehydration of corn kernels to about 4% moisture content required about 1.5 hours in the vacuum oven, and about 5–7 hours in the hot-air oven or stream of hot air.

Results and Discussion

Appearance of Kernels after Spraying. Ten seconds or less after the freshly cut corn surfaces are sprayed with the blue cobalt chloride solution, the relatively moist portions of the partially dehydrated corn turn pink, while the dried portions retain the original blue of the solution, or are green, probably due to a blending of the yellow corn color with the blue of the solution (Fig. 1). Each of the kernels represented in Fig. 1 was taken from a composite of ten or more kernels, and starting from the left, each kernel is more dehydrated than the preceding one. The corn kernels represented have been sliced approximately in half, longitudinally, after having been partially dehydrated by means of a stream of hot air or in a hot-air oven at either 110° or 130°F. (43° or 54°C.). The darkened areas of each kernel actually appear pink in the cobalt chloride spray test, and represent portions of the kernel where there is moisture in a concentration greater than about 4%. The light areas actually appear greenish-blue in the test, and indicate portions of the kernel where the moisture content is below about 4%. Note how, in Fig. 1, Section 1, as the kernel approaches complete dehydration the remaining moisture has

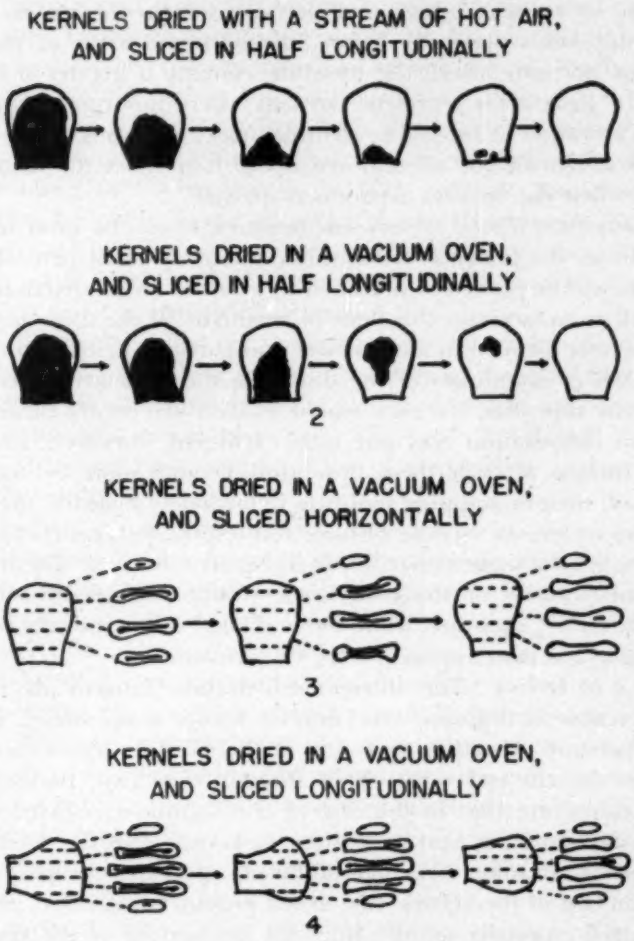
KERNELS DRIED WITH A STREAM OF HOT AIR,
AND SLICED IN HALF LONGITUDINALLY
KERNELS DRIED IN A VACUUM OVEN,
AND SLICED IN HALF LONGITUDINALLY
2
KERNELS DRIED IN A VACUUM OVEN,
AND SLICED HORIZONTALLY
3
KERNELS DRIED IN A VACUUM OVEN,
AND SLICED LONGITUDINALLY
4

Fig. 1. The darkened areas in the above diagrams represent regions in partially dehydrated corn kernels where moisture is concentrated. The unshaded areas contain less than 4%. In each row, each kernel is more dehydrated than the one to its left.

diffused to the surface cut from the ear, i.e., the lower surface on the diagram. In Section 2, however, note the different manner of drying. These diagrams represent kernels dried in a vacuum oven. As the kernel becomes more dehydrated (from left to right) the remaining moisture is found more or less in the center of the kernel.

Sections 3 and 4 are diagrams of kernels partially dried in a vacuum oven, which picture essentially the same moisture pattern as in Section 2, but with different slicings of the kernels, allowing one to obtain a three-dimensional concept. The kernels were cut at the points

indicated by the broken lines, and each slice was turned so as to expose the freshly cut surface. As before, the darkened areas of the slice represent portions where the moisture content is greater than 4%, while the light areas represent portions where moisture is less than 4%. Dehydration of kernels to identical stages by means of the hot-air oven or stream of hot air required about four times the amount of time as when the vacuum oven was employed.

Sensitivity of Test. At very low moisture levels the spray test will not indicate the presence of moisture, and yet a small percentage of moisture will be present as indicated by a vacuum-oven determination. In an effort to ascertain this limit of sensitivity of the spray test, corn samples were blended in an Osterizer and partially dried as thin films in the lids of aluminum drying dishes on the assumption that, in a sufficiently thin film, the corn would tend to dry evenly throughout. Uniform dehydration was not quite achieved, however, since the upper surface of even these thin films became more or less case-hardened, thereby trapping moisture immediately beneath, making it necessary to remove sections of films and test the lower surfaces. However, the results suggest that the darker-shaded areas in the diagrams represent portions of the corn kernel containing greater than approximately 4% moisture, while the unshaded areas represent portions containing less than approximately 4% moisture.

Effect of Drying. The different dehydration patterns may be due to differences in diffusion rate, interior kernel temperature, and external pressure. Assuming that most of the moisture leaves the kernel through the cut end rather than through the dense pericarp, one might conjecture that in the case of the vacuum-oven-dried kernels (Fig. 1, Section 2), as heat penetrates the kernel from the outer edges, the moisture in these areas starts diffusing (perhaps as vapor) toward the open end of the kernel. Due to the pressure differential, moisture is removed especially rapidly from the cut surface of the kernel, so rapidly, in fact, that moisture from the center of the kernel does not diffuse fast enough (at the lower temperature found at the center) immediately to fill the gap left by removal of moisture at the cut surface of the kernel. In the case of kernels dried in the hot-air oven or stream of hot air (Fig. 1, Section 1), one might conjecture that, due to the comparative slowness of removal of moisture from the cut surface of the kernel, the moisture from the center of the kernel becomes sufficiently heated so that diffusion of moisture from the center at least equals the rate of evaporation at the cut surface, and therefore the final moisture is found at the cut surface of kernels.

It was thought that casehardening of the cut surface of a kernel

might have sealed this exit against passage of moisture, and thus one would expect that moisture might be found near this sealed surface as long as moisture remained in the kernel. Casehardening might explain the results for dehydration at atmospheric pressure (Fig. 1, Section 1), but this hypothesis did not seem to explain the results observed with vacuum-oven-dried kernels (Section 2), the same temperature (100°C.) being used for both methods. Previously, casehardening had been found to occur in the vacuum-oven drying of blended corn samples, so that one could not assume that this effect did not occur in vacuum-oven-dehydrated kernels, and yet did occur in kernels dried at atmospheric pressure.

To discover whether the temperature at which dehydration was carried out had any effect on the sensitivity of the spray test at low moisture levels, blended samples of corn were dried in a hot-air oven at three different temperatures, 90°, 110°, and 130°F. (32°, 43°, and 54°C.) There was no discernible effect of temperature of dehydration upon the results.

Effect of Time of Observation. Previously it was mentioned that some observations were made 30 seconds after the corn was sprayed with the cobalt chloride–methyl alcohol solution. When observations were taken after this arbitrary interval, as compared with the 10-second observation, the test appeared to be capable of detecting moisture in blended corn samples at concentrations as low as 1.5%, as compared with 4% for the 10-second period. If the period from spraying to observing were extended several minutes, an originally pink area (indicating moisture) would extend over the entire sprayed surface, or an entirely blue-green surface (indicating no detectable moisture) would eventually turn entirely pink. These phenomena were considered to be due, at least in part, to interference from moisture in the air (the test was conducted under humid weather conditions), to diffusion of the solution over the sprayed surface of the corn kernel, and to evaporation of the methyl alcohol from the solution. Some moisture from the air might be trapped by the methyl alcohol, which has an affinity for water, and in addition, a lowering of temperature caused by evaporation of the methyl alcohol at the surface of the kernel might cause condensation of moisture. A 10-second interval after spraying and before recording observations was selected as being more desirable than immediate recordings, since it was thought that the 10-second period was necessary in determinations on corn with low moisture content to permit sufficient mixing of water in the corn with the cobalt chloride.

Error of the Method. Some moisture from the air was undoubtedly

absorbed by the dehydrated corn kernels while they were being cut and pinned in the field of the microscope, but this was not thought to be a serious error. Temperature, which, when elevated, increased the rate of color change in measurements of plant transpiration with cobalt chloride paper (4), was not investigated in the present study other than by observations at air temperatures of 70° and 92°F.; no noticeable effect on the test was detected. Temperature effects caused by evaporation of methyl alcohol at the sprayed surfaces were not studied.

The combined effect of absorbed moisture from the air and evaporation of the methyl alcohol probably caused a very slight error that would make the spray test appear more sensitive at the low-moisture limit than actually was the case. That is, the moisture which the cobalt chloride–methyl alcohol solution and dehydrated corn take from the air, and the evaporation of some of the alcohol which keeps the cobalt chloride dehydrated, would tend to allow some rehydration of the cobalt chloride, whereas the rehydration was calculated to occur only as a result of moisture from the corn.

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GROWTH-PROMOTING SUBSTANCES IN WHITE SUGARS FROM DIFFERENT SOURCES OF MANUFACTURE AND THEIR EFFECT ON FERMENTATION[1]

ARCHER C. WILCOX[2], BYRON S. MILLER, AND MAX MILNER

ABSTRACT

Small quantities of growth-promoting substances (vitamin B-complex) including thiamine, niacin, pyridoxine, pantothenic acid, and biotin, were found in dextrose sugar from corn processing and in sucrose sugar from beet processing; but apparently were absent in sucrose sugars from cane sugar processing. Their presence in fermenting solutions resulted in an increased rate of fermentation over that in the vitamin-free medium but did not affect the rate of fermentation in bread doughs.

The literature pertaining to the vitamin requirements of baker's yeast *(Saccharomyces cerevisiae)* during dough fermentation has been reviewed recently by Rice and Ramstad (11). If the yeast is not supplied with the proper amounts of the required vitamins, the fermentation process will be retarded (3). Most strains of *S. cerevisiae* are unable to synthesize biotin and some cannot produce pantothenic acid and pyridoxine. The vitamin requirements of yeast in bread doughs are usually met adequately by the flour although biotin is completely lacking.

Hall, Paine, and Fabian (7) found that sucrose from different sources of manufacture contained growth-promoting substances (members of the vitamin B complex) as impurities adsorbed on the surface of the crystals. The presence of these substances in sucrose as well as in dextrose might affect the rate of fermentation in yeast-leavened baked products. The purpose of this investigation, therefore, was to determine by microbiological assay methods the presence and concentration of growth-promoting substances in a number of commercial sugars, their effects on anaerobic and aerobic fermentation by the yeast, and their influence on the rate of fermentation in bread doughs.

Materials and Methods

Seven samples of dextrose and sucrose were analyzed for the presence of growth-promoting substances. These included dextrose ob-

[1] Manuscript received December 16, 1952. Cooperative investigations between the Department of Flour and Feed Milling Industries, Kansas State College, and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture. Contribution No. 228, Department of Flour and Feed Milling Industries, Kansas Agricultural Experiment Station.

[2] Present Address: Red Star Yeast and Products Co., Milwaukee, Wisconsin.

tained from corn processing, three sucrose samples from sugar beets, and three sucrose samples from sugar cane.

The microbiological assays were conducted with *Leuconostoc mesenteroides* (Strain P-60, American Type Culture Collection No. 8042), employing a modification of the methods of McMahan and Snell (9) and Gaines and Stahly (6). The composition of the modified basal medium and the vitamin concentrations which were used in obtaining the standard curves are presented in Table I. Assay procedures were carried out according to the method given by Snell (12).

TABLE I

COMPOSITION OF BASAL MEDIUM FOR MICROBIOLOGICAL ASSAY WITH L. MESENTEROIDES

Part A (required for all assay tubes) Constituent	Concentration
	g./tube
Acid-hydrolyzed vitamin-free casein	0.50
Anhydrous sodium acetate	0.60
Sugar (dextrose or sucrose)	1.00
l-Cystine hydrochloride	0.01
dl-Tryptophan	0.01
Adenine, Guanine, and Uracil	0.01
K_2HPO_4	0.05
KH_2PO_4	0.05
$MgSO_4 \cdot 7H_2O$	0.02
$FeSO_4 \cdot 7H_2O$	0.001
$MnSO_4 \cdot 7H_2O$	0.001
Part B (required for standard curves only) Constituent	Concentration Range
	μg./tube
Thiamine	0.0 to 5 $\times 10^{-1}$
Niacin	0.0 to 5 $\times 10^{-1}$
Pantothenic acid	0.0 to 5 $\times 10^{-1}$
Pyridoxine	0.0 to 5 $\times 10^{-1}$
Biotin	0.0 to 1 $\times 10^{-1}$
Folic acid	0.0 to 2.5 $\times 10^{-3}$
p-Aminobenzoic acid	0.0 to 1.25 $\times 10^{-4}$

The effect of growth-promoting substances on anaerobic and aerobic fermentation by the yeast cell was determined manometrically by means of a "Precision" Warburg constant-volume respirometer utilizing the standard single-arm Warburg flasks. The techniques used and the theory involved are described by Dixon (5) and Umbreit, Burris, and Stauffer (14). The complete medium used in all determinations is presented in Table II. All components used were free from vitamin contamination, with the exception of the commercial sugars.

TABLE II

MEDIUM USED IN ALL MANOMETRIC DETERMINATIONS

Constituent	Quantity	Dissolved in Distilled Water
Part A (required for all flasks)	*mg.*	*ml.*
Yeast (Standard Brands Inc.)	2.5	1.00
Sugar (sucrose or dextrose)	30.0	0.55
Sodium citrate (pH = 5.5)	8.65	0.50
Asparagine	10.03	0.50
Salts (see below)	5.80	0.50
Vitamins (see below)	..	0.40
		Total 3.45
Part B (required for all flasks)		
Salts	*mg./flask*	
$NaH_2PO_4 \cdot 7H_2O$	3.01	
$MgSO_4 \cdot 7H_2O$	1.99	
KCl	0.80	
Part C (required for complete medium only)		
Vitamin	*μg./flask*	
Thiamine	16.0	
Niacin	16.0	
Pyridoxine	16.0	
Calcium pantothenate	16.0	
i-Inositol	32.0	
Biotin	0.30	
p-Aminobenzoic acid	trace	
Folic acid	trace	

The yeast used in all determinations was compressed bakers' yeast (Standard Brands, Inc.). The yeast suspension was prepared by dispersing the required quantity of fresh yeast in 120 ml. of distilled water, centrifuging, and discarding the supernatant. The cells were washed six times with physiological saline to remove any substrate or growth factors, washed twice with distilled water to remove the saline solution, and resuspended in 120 ml. of distilled water. The proper dilution was made of the final suspension and 1 ml. of the dilution was used as the inoculum for the reaction flasks.

All determinations were carried out at 30° C. ± 0.2° C. at a shaking rate of 110 oscillations per minute. Flask constants, gas evolution rates, and total quantities of gases consumed or evolved were calculated according to the methods of Umbreit, Burris, and Stauffer (14). Ethyl lactate plus crystal violet as a coloring agent was used as the manometer fluid (14).

Anaerobic fermentation rates and total carbon dioxide evolutions were measured under an atmosphere of nitrogen which had been freed of traces of oxygen by passing commercial tank nitrogen through a solution of alkaline pyrogallol before the reaction flasks were

flushed. The complete medium, including yeast cells minus the sugar solutions, was placed in the main compartments of the flasks. The sugar solutions were carried in the sidearms of the flasks to facilitate starting the measurements at zero time.

Aerobic fermentation rates and total oxygen uptake were carried out in an atmosphere of oxygen. The only change in the general procedure (14) was that potassium hydroxide solution was placed in the center-wells instead of acid (anaerobic measurements) to absorb the carbon dioxide liberated.

The effect of growth-promoting substances present in the sugar samples on the rate of fermentation in bread doughs was determined by means of gas production tests using the standard method for pressuremeters as described in Cereal Laboratory Methods (2). To each pressuremeter was added 10 g. flour, 0.3 g. yeast suspended in 5 ml. of water, and 0.6 g. of the respective sugar samples in 5 ml. of water.

Results and Discussion

The presence of small quantities of growth-promoting substances in white sugars from different sources of manufacture is shown by the data presented in Table III. These data corroborate the work of Hall, Paine, and Fabian (7), which showed that growth-promoting substances are present in sucrose sugars. The dextrose sample from corn processing and all the sucrose samples obtained from beet sugar processing were shown to contain growth-promoting substances, whereas the sucrose samples obtained from cane sugar processing were found to be essentially free of growth-promoting substances.

TABLE III

EFFECT OF GROWTH-PROMOTING SUBSTANCES PRESENT IN VARIOUS SUGAR SAMPLES ON LACTIC ACID PRODUCTION BY L. MESENTEROIDES

Sugar	Total Lactic Acid Production[1] Assay Numbers 1	2	3	4	5	6	Average Values, Lactic Acid	Maximum Possible Growth
	ml.	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*%*
Vitamin-free dextrose	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
Commercial dextrose	0.60	0.55	0.60	0.53	0.40	0.57	0.54	20.0
Cane sugar, 1, 2, 3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
Beet sugar, 1	0.40	0.00	0.30	0.48	0.30	0.38	0.37	13.5
Beet sugar, 2	0.40	0.23	0.30	0.43	0.33	0.41	0.37	13.1
Beet sugar, 3	0.40	0.30	0.35	0.48	0.31	0.37	0.37	13.3

[1] Milliliters lactic acid equivalent to 0.10 N sodium hydroxide/g. of sample.

The effect of increasing concentrations of growth-promoting substances contained in a commercial dextrose sugar on lactic acid production by *L. mesenteroides* is presented in Table IV. The concentration of growth-promoting substances increased with increasing

TABLE IV

CONCENTRATION OF GROWTH-PROMOTING SUBSTANCES IN COMMERCIAL DEXTROSE SUGAR MEASURED BY LACTIC ACID PRODUCTION USING L. MESENTEROIDES

Dextrose Concentration		Total Lactic Acid Production[1]
g./10 ml. of solution		*ml.*
0.0025	Control (vitamin-free dextrose)	0.00
0.0025	Commercial dextrose	0.60
0.005	Commercial dextrose	2.30
0.010	Commercial dextrose	3.45
0.10	Commrecial dextrose	3.40
0.50	Commercial dextrose	3.00
1.00	Commercial dextrose	2.70
2.00	Commercial dextrose	2.80
5.00	Commercial dextrose	2.20
10.0	Commercial dextrose	1.20

[1] Milliliter lactic acid equivalent to 0.1 N sodium hydroxide.

sugar concentration, but the increase was not linear. This may be due to an osmotic effect of the sugar on the bacterial cell.

The results of the growth-response method for measuring the presence and concentration of the individual growth-promoting substances are presented in Table V. These data indicate that the con-

TABLE V

APPROXIMATE VITAMIN CONCENTRATIONS IN SUGARS FROM DIFFERENT SOURCES OF MANUFACTURE AS DETERMINED BY GROWTH RESPONSE OF L. MESENTEROIDES

Sugar	Approximate Vitamin Concentrations				
	μg./g. of sample				
	Thiamin	Niacin	Pyridoxine	Pantothenic Acid	Biotin
Vitamin-free dextrose	0.0000	0.0000	0.0000	0.0000	0.000000
Commercial dextrose	0.0020	0.5500	0.7500	0.0090	0.000033
Beet sugar, 1	0.0029	0.5800	0.2000	0.0900	0.000021
Beet sugar, 2	0.0030	0.6500	0.6700	0.0190	0.000079
Beet sugar, 3	0.0046	0.5400	0.6500	0.0270	0.000025

centration of growth-promoting substances present in some white sugars was sufficient to warrant further investigation by means of the Warburg respirometer.

The effects of growth-promoting substances, previously identified as B-vitamins in the several sugars, on anaerobic and aerobic fermentation as determined with yeast cells in the Warburg respirometer, are presented in Fig. 1. Commercial dextrose without added vitamins

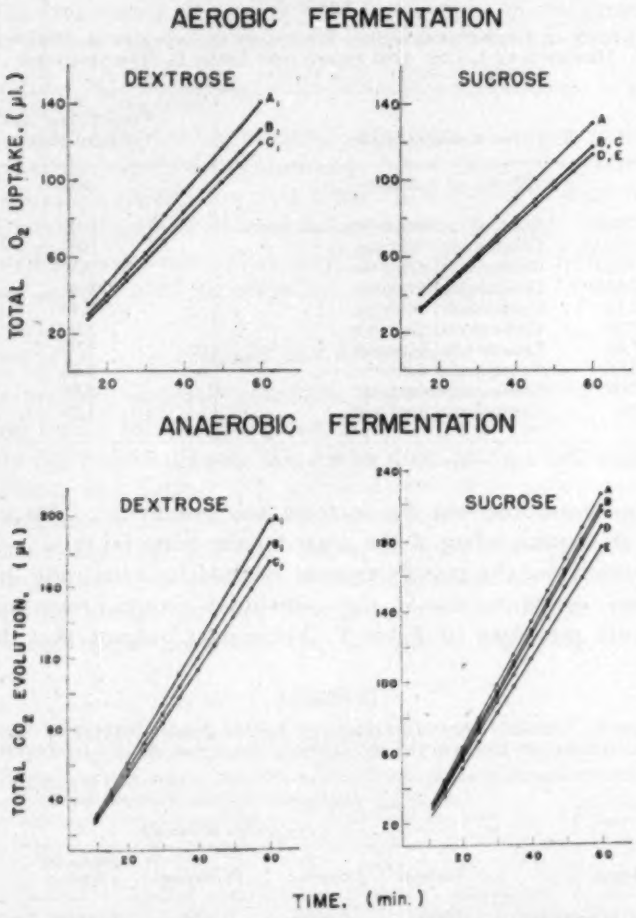

Fig. 1. Effect of growth-promoting substances present in commercial dextrose and sucrose on aerobic and anaerobic fermentation by the yeast cell. **Left:** A_1, vitamin-free dextrose plus added vitamin medium; B_1, commercial dextrose; C_1, vitamin-free dextrose. **Right:** A, vitamin-free sucrose plus added vitamin medium; B, commercial sucrose, 3; C, commercial sucrose, 2; D, commercial sucrose, 1; E, vitamin-free sucrose.

gave an increase in total carbon dioxide production, under anaerobic conditions, over that of the vitamin-free dextrose. Since all other constituents of the medium were vitamin-free, these results indicate

that the presence of growth-promoting substances in the sugar caused the increase in carbon dioxide production. However, the content of growth-promoting substances of the commercial sugar used was either insufficient or incomplete for maximum carbon dioxide production. The results for the sucrose samples were similar to those obtained with the dextrose samples, and show that the presence of growth-promoting substances affects anaerobic fermentation. The response to the addition of a complete vitamin medium to the commercial samples was greatest in sucrose sample 3, followed by that of samples 2 and 1.

The effects of growth-promoting substances under aerobic conditions were similar to those obtained under anaerobic conditions. No attempt was made to determine the effect of individual growth factors.

Although experimental conditions used in the present study for the determination of the effect of growth-promoting substances on fermentation were not identical to those used by other workers (1, 4, 8, 10, 13, 15), the fermentation quotients indicated generally good agreement among the various data.

The influence of growth-promoting substances in commercial dextrose and sucrose on gas production is shown in Table VI. These

TABLE VI

EFFECT OF GROWTH-PROMOTING SUBSTANCES IN SUGARS ON GAS PRODUCTION IN DOUGHS AT 30°C.

Time	Sugar Sample			
	Vitamin-Free Dextrose	Commercial Dextrose	Vitamin-Free Sucrose	Commercial Sucrose
hr.	*mm. Hg./hr.*	*mm. Hg./hr.*	*mm. Hg./hr.*	*mm. Hg./hr.*
0.5	40.7	39.0	36.7	34.7
1.0	128.3	126.0	126.7	123.7
2.0	169.6	167.0	168.3	169.3
3.0	162.3	158.3	162.0	162.0
4.0	127.7	132.0	146.7	149.3
5.0	140.7	139.0	137.6	137.3

results suggest that the quantities of growth-promoting substances contributed by the sugar do not have an appreciable effect on the rate of fermentation in bread doughs.

Acknowledgment

Acknowledgement is given to Corn Products Sales, Inc., New York, N. Y., for financial assistance in making this project possible.

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EMULSIFIERS IN PREPARED CAKE MIXES[1]

H. H. Favor, L. B. Crossland, and H. K. Beasley[2]

ABSTRACT

Monoglyceride- and polyoxyethylene-type emulsifiers increased the volume of white cake at most shortening levels and permitted the use of higher shortening levels for maximum cake volume. Combinations of both types of emulsifier produced more volume than could be obtained with either emulsifier by itself.

The importance of emulsifiers in certain baked products was first called to the baking industry's attention when emulsifying shortening was introduced. Monoglyceride-type emulsifiers in shortening permitted the production of cake containing higher proportions of sugar, fat, and water than had earlier been used, as is indicated in the patent literature for the past 20 years. Carlin (1) in 1951 called attention to their use in prepared cake mixes. The introduction of the sorbitan partial esters and their polyoxyethylene derivatives provided emulsifiers with much wider range in hydrophile-lipophile balance (2).

In the authors' experience, the problems of properly processing batters from retail prepared cake mixes are different from those of making batters from baker's ingredients with baker's mixers. As might be expected, emulsifiers play somewhat different roles in the two types of cake batters. In the bakery, batters get vigorous and extended agitation with consequent incorporation of many air bubbles. Ingredient and batter temperatures, as well as batter specific gravities, are carefully controlled in all well managed bakeries. The degree of control a baker can maintain over the quality of his product by specifying the mixing procedure must be accomplished by the prepared-mix manufacturers through formulation. The baker can incorporate an amount of air that he finds by experience to be desirable. In contrast, retail prepared mixes may be mixed by hand, or by any one of a number of mixers, with little if any attention given to temperature or batter specific gravity. Prepared mixes must, therefore, be formulated so that it is not necessary to incorporate given quantities of air to obtain a good product.

Lightness, widely recognized as desirable in many types of cakes, is markedly influenced by emulsifiers which also affect grain, tender-

[1] Manuscript received June 26, 1952. Presented at Annual Meeting, American Association of Cereal Chemists, April, 1952, Dallas, Texas.

[2] R. T. Vanderbilt Co., Inc., East Norwalk, Connecticut.

ness, crust appearance, and symmetry. The present paper deals with a study of the effect of emulsifiers on the specific volume of cakes made from prepared mixes. The studies involve a wide range of shortening levels without emulsifier and with various levels of monoglyceride and polyoxyethylene emulsifiers recommended for prepared mixes.

Materials and Methods

Prepared Mix Formula. A prepared mix formula for white cake for household use requiring only the addition of milk was used (Table I). The flour was a high-grade commercial cake flour milled from soft red wheat; protein content 8.50%, ash content 0.32%, pH 4.9.

TABLE I

WHITE CAKE PREPARED MIX FORMULA

Ingredients	Percentage of Mix	Percentage of Flour
Flour	36.60	100.00
Sugar	47.50	130.00
Shortening	11.00	30.00
Emulsifier	0.24	0.66
Salt	1.10	3.00
Baking powder	1.82	5.00
Dried egg white	1.74	4.75

The sugar was extra-fine granulated. Two all-hydrogenated vegetable oil shortenings were used; one contained 5% of mono- and diglycerides, the other did not. The salt was table salt and the baking powder was a commercial one containing sodium acid pyrophosphate, monocalcium phosphate, calcium lactate, sodium bicarbonate, and starch. Two types of emulsifier were used. One was a superglycerinated fat such as is used in emulsifying shortening for prepared cake mixes, referred to as monoglyceride. The other was a polyoxyethylene type and consisted of a mixture of polyoxyethylene sorbitan monostearates, with an average of 6 moles of ethylene oxide per mole of sorbitan monostearate, referred to hereafter as poly emulsifier.

A preliminary set of experiments was designed to determine a reasonable level of shortening for use in conjunction with the selected levels of other ingredients. Emulsifying and non-emulsifying shortenings, each with and without polyoxyethylene emulsifiers, were studied.

The level of shortening selected was 11% based on the total weight of dry mix. Regular shortening, emulsifying shortening, regular

shortening plus poly emulsifier, and emulsifying shortening plus poly emulsifier were investigated through the range 0–17.5%.

At 11% shortening, levels of monoglyceride emulsifiers (replacing an equal amount of shortening) were studied through the range of 0 to 1.54% based on the weight of mix. This corresponds to 0–14% of monoglyceride by increments of 2% on the weight of the shortening. As shortening levels were varied, scaling weights of the prepared mix were adjusted so that the ratio of liquids to flour was constant.

Preparation of Batter. A weight of prepared mix containing 150 g. of flour was scaled into the large bowl of a Sunbeam Model 10 Mixmaster. One-half cup of milk was added and the mixture was blended 0.5 minute at first speed (250 r.p.m.), then beaten 2 minutes at third speed (350 r.p.m.). Two more additions of milk, 0.25 cup each, were made by blending 0.25 minute at first speed, followed by beating 1 minute at third speed. Batter temperatures were 70° F. Fourteen ounces of batter were scaled into 8″ x 1.25″ round aluminum cake pans. Sides were greased and paper disks were used on the bottoms of pans. Cakes were baked 21 minutes at 375° F., removed from pans immediately after baking, and cooled on wire racks for 1 hour. Volumes were taken by rape seed displacement and are expressed as specific volume (cc. per g.).

Photomicrographs. To study the effects of emulsifiers on batter properties in more detail, batters were prepared in the same manner as for the baking studies, with the exception that a yellow fat-soluble dye was added to the shortening. These batters were then observed under a microscope immediately after mixing. Photomicrographs were

Results and Discussion

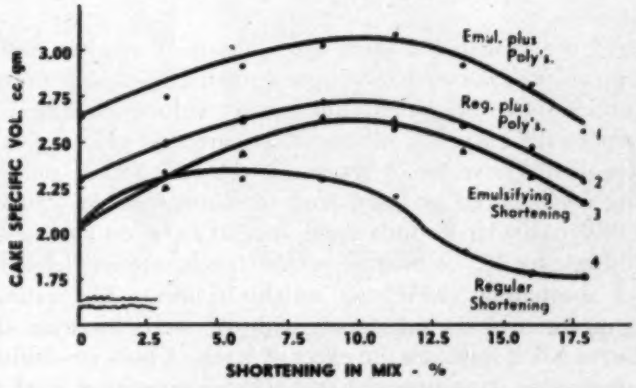

Fig. 1. The influence of shortening levels on the volume of white cakes made from prepared mix.

taken of batters with and without shortening, containing no emulsifier, with monoglyceride emulsifier, and with poly emulsifier.

Figure 1 shows the effect of increasing amounts of two shortenings on specific cake volume with and without poly emulsifier. The lower curve (No. 4) indicates that with the small amount of aeration produced by mixing procedures recommended for prepared mixes, volume is poor when shortening with no emulsifier is used for this type of cake. Curve No. 3 indicates that emulsifying shortening produces appreciably better cake volume, especially at shortening levels near 11% on the weight of this mix. Curve No. 2 is similar to Curve No. 3 and indicates that the ploy emulsifier, when used with non-emulsifying shortenings, produces good cake volume. Curve No. 1 compared to Curve No. 3 indicates the response of emulsifying shortening to poly emulsifier. Obviously, the cake volumes permitted by this combination are very good and indicate that emulsifying shortenings may be preferred to regular shortening for use with poly emulsifier.

For those tests where poly emulsifier was used, the percentage of ester based on weight of flour was constant and at a shortening level of 11% amounted to 0.24% of the weight of mix. Of the 32 shortening and emulsifier combinations studied, shortening plus emulsifier consistently produced greater cake volume; the one exception was when the shortening level was extremely low. In three of the four series of tests it was found that maximum volume was obtained when the shortening levels were nearer 11% of the mix. For subsequent work, 11% of shortening was used because in addition to best volume, better grain and somewhat improved eating quality were often obtained near this level.

With 11% of shortening based on the weight of prepared mix, the effect of increasing levels of emulsifier with various shortenings was then studied.

In Fig. 2 the broken line from the ordinate at a specic volume of 2.25 represents the cake volume obtained when 11% of shortening containing no emulsifier was used. The greater volume indicated by all other parts of the curves is, of course, imparted by the one or more emulsifiers used. Curve No. 3 represents the effect when portions of shortening are replaced by increments of monoglyceride type emulsifier. For this particular formula there appears to be no volume advantage in using over 4% of monoglyceride-type emulsifier based on the weight of shortening (0.44% on weight of mix). The eating and handling qualities of the cake made with 6% were, however, slightly better. Curve No. 2 indicates the effect of levels of poly emulsifier with regular shortening. It is apparent that there is no critical level. Curve

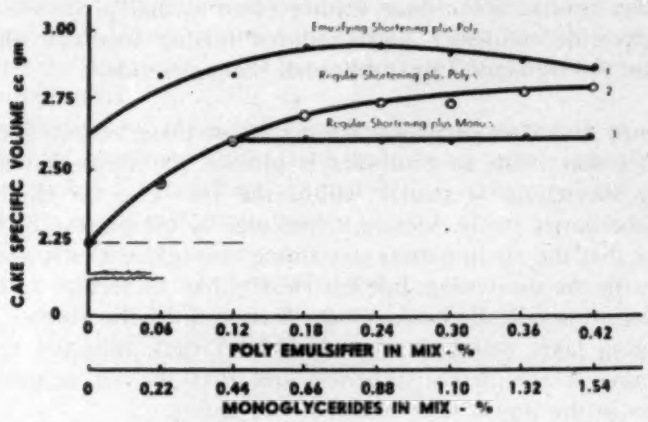

Fig. 2. The effect of emulsifier levels on the volume of white cakes from prepared mix.

No. 1 shows the effect of levels of poly emulsifier with emulsifying shortening. For this series the amount of monoglyceride emulsifier is constant. Only the level of poly emulsifier varies. It is apparent that the effects of the two types of emulsifier are supplementary. Together they will do what either alone cannot do in any quantity. It is also apparent that a considerably smaller quantity of the poly

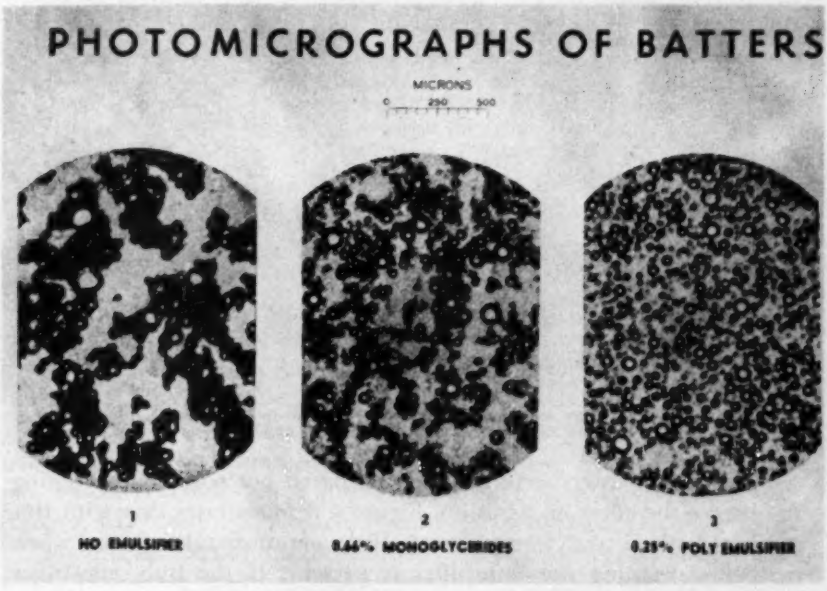

Fig. 3. The influence of emulsifiers on incorporation and dispersion of air in cake batters containing shortening.

emulsifier produced the same volume effect as that produced by the monoglyceride emulsifier, and produced further increases when no response was obtained from additional monoglyceride.

Figure 3 is a set of photomicrographs of these batters. Section 1 indicates that when no emulsifier is present, the air in batters containing shortening is clearly within the fat. The fat clumps are large, the batter curdy. Section 2 indicates by the pattern of bubble clusters that the air in batters containing monoglycerides is also identified with the shortening, but less clearly than in Section 1. The fat particles are small, the batter glossy. Section 3, by the absence of any shortening lakes and lack of air-bubble clusters, indicates that the shortening is completely dispersed and that the air is present as bubbles in the liquid. The batter is very glossy.

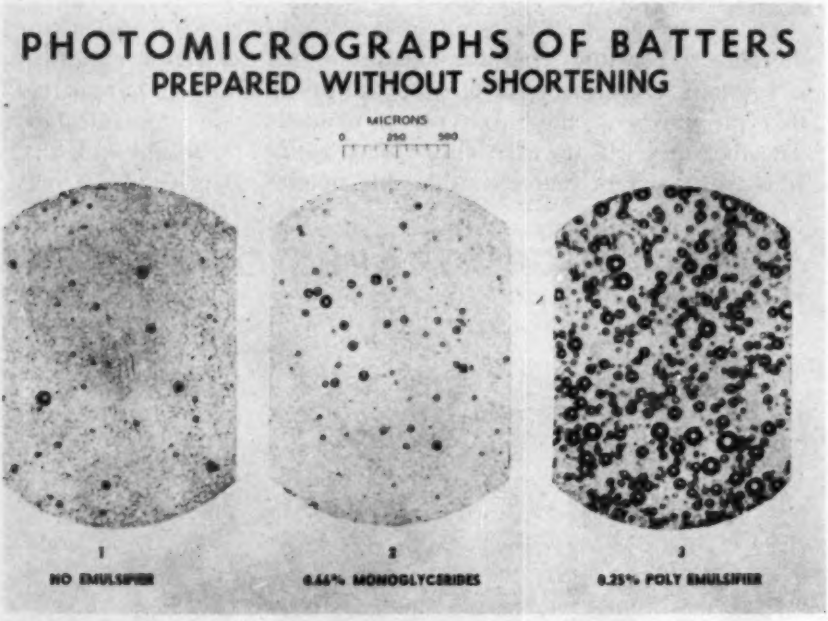

Fig. 4. The influence of emulsifiers on incorporation of air in cake batters containing no shortening.

Additional batters were similarly prepared but without shortening to observe the effect on aeration. Figure 4 demonstrates that with this mixing method and formula very little aeration takes place when neither shortening nor emulsifier is present. If the only emulsifier used is monoglyceride, air is not incorporated. If poly emulsifier is

present, considerable air enters the batter. The absence of shortening in the last set of batters reduced the viscosity. The lower viscosity accounts for the difference in the air content of batters containing the poly emulsifier (cf. Fig. 3, Fig. 4).

Thus, because of increased volume and improvements in other factors of cake quality resulting from their use, it appears that monoglycerides and poly emulsifiers are useful in meeting the specialized requirements of dry-mix formulations.

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THE DISTRIBUTION OF PROTEIN IN THE MAIZE KERNEL IN COMPARISON WITH THAT IN WHEAT[1,2]

J. J. C. HINTON

ABSTRACT

Flinty maize has been dissected by hand into seven anatomical regions and the distribution of protein between these regions determined. Seventy percent of the total protein was found in the endosperm, in which a concentration gradient exists from the outer to the inner layers. The distribution resembles that previously reported for wheat except that in maize the aleurone layer, which is smaller, contains less of the total protein than in wheat, and the scutellum, which is larger, contains more.

The differences in the chemical composition of the various anatomical regions of cereal grains has always been of interest to cereal chemists and, apart from information obtained by the analysis of mill streams, the composition of precise anatomical regions has been studied on hand-dissected material. This work appears to have been initiated by Hopkins, Smith, and East in 1903, who worked out the distribution of protein, fat, ash, and carbohydrate in the maize kernel (10). About the same time, by similar methods, Cobb investigated the distribution of gluten in wheat (2). The distribution of protein in wheat has also been more recently investigated by Morris, Alexander, and Pascoe (11) and by the author (8), while that of the total ash and some of its constituents was also examined by Morris, Alexander, and

The present communication reports the results of work on the dis-

[1] Manuscript received August 23, 1952.
[2] Contribution from the Research Association of British Flour Millers, St. Albans, England.

Pascoe. The distribution of thiamin in wheat and rice has been studied by the author (8, 9), and recently it was shown that 60% of the niacin in corn and 82% in wheat is concentrated in the aleurone layer (7).

Microtome sectioning methods replaced hand dissection in an investigation by Engel (4, 5, 6) of the location of some enzymes in wheat, rye, and barley, while hand-dissected material was again used by Earle, Curtis, and Hubbard (3) in a study of differences in chemical composition between component parts of the corn kernel.
tribution of protein in hand-dissected fractions of flint maize and compares them with similar results previously reported for wheat.

Material and Methods

The flint variety Sibthorpe was used as material. This variety was originated by Prof. G. E. Blackman of the University of Oxford, England, as a selection from German material of unknown genetic constitution. It is very early maturing, suitable in this respect for growing under English conditions (1).

The dissection was carried out by hand, using a low-power binocular microscope and normal dissecting needles and knives. The parts separated were pericarp, aleurone layer, three regions of the endosperm, embryo, and scutellum.

The pericarp fraction consisted of epidermis and mesocarp together with portions of cross-layer and tube cells, the way in which the division of the latter layers occurred not being precisely determined. This fraction, the embryo, and the scutellum were removed with the kernel in an air-dry condition. The aleurone layer included the seed coats and fragments of the tube-cells and cross layer. Its removal was facilitated by directing onto it a stream of warm moist air which kept it pliable and capable of being handled without breakage. After removal of these fractions the horny part of the endosperm was softened by placing the kernel in a moist chamber for two or three days. Warm, moist air was again directed onto it during the dissection of the outermost layer, one or two cells in thickness, described as endosperm 1. The loose floury portion in the center was then removed as endosperm 3 and the remainder of the horny vitreous portion is described as endosperm 2.

The proportion of the kernel which each fraction represents was determined by a complete dissection of three typical kernels. The samples on which protein was determined were a mixture of representative portions from ten kernels.

The water content of the endosperm under the laboratory conditions at the time was approximately 14%, while that of embryo and scutellum was about 10%.

Nitrogen was estimated by the micro-Kjeldahl method using copper selenite as catalyst. Determinations on fractions, aleurone layer, and endosperm 1 were in triplicate, the remainder in duplicate.

Results and Discussion

The results are presented in Table I, where they are compared with previously published figures for wheat (var. Vilmorin 27) obtained in the same way (8).

TABLE I

PROTEIN DISTRIBUTION IN MAIZE COMPARED WITH THAT IN WHEAT

	Proportion of the Kernel		Protein Content (N × 6.25)[1]				Proportion of the Total Protein of Kernel	
	Maize	Wheat	Maize		Wheat		Maize	Wheat
	%	%	%	*per 100 g. kernel*	%	*per 100 g. kernel*	%	%
Pericarp	6.5	8.0	3.0	0.2	4.4	0.3	2.2	4.0
Aleurone layer	2.2	7.0	19.2	0.4	19.7	1.4	4.7	15.5
Endosperm 1	3.9	12.5	27.7	1.1	13.7	1.7	11.9	19.4
Endosperm 2	58.1	12.5	7.5	4.4	8.8	1.1	48.2	12.4
Endosperm 3	17.6	57.5	5.6	1.0	6.2	3.6	10.9	40.7
Embryo	1.1	1.0	26.5	0.3	33.3	0.3	3.2	3.5
Scutellum	10.6	1.5	16.0	1.7	26.7	0.4	18.9	4.5
Whole Grain	100.0	100.0	9.0	9.1	8.7	8.8	100.0	100.0

[1] 14% moisture content basis.

The subdivisions of the wheat endosperm are (1) immediately within the aleurone layer and one or two cells in thickness; (2) immediately within 1 and of similar dimensions; and (3) the main central part.

The protein found in 100 g. of dissected kernels is in good agreement with that in 100 g. of whole corn. Although the present results cannot be compared fully with those of Hopkins, Smith, and East (10), as the dissection was different in the two cases, the pattern of distribution of protein is broadly similar in the two investigations. One of the samples used by Hopkins, *et al.* contained 9.9% protein, comparable to that used in the present work, and was divided by them into tip cap, hull, horny gluten, horny starch, crown starch, tip starch, and germ.

Tip cap and hull together correspond to pericarp of the present work; they comprised 6.6% of the kernel, contained 6% of protein,

and about 3.6% of the protein of the whole kernel, compared with 6.5%, 3%, and 2% respectively for the pericarp.

The horny gluten probably compares with endosperm 1 together with the aleurone layer. It comprises 7.7% of the kernel and contains about 19% of protein and 22.5% of the protein of the kernel, compared with 6%, 24%, and 16.5% respectively for the combined fractions.

The horny starch probably compares with endosperm 2 but the samples were evidently different in their degree of mealiness, since it comprises 37% of the kernel compared with 58% for endosperm 2. The protein content is similar in each case, 8% and 7.5% respectively, while the proportion of the total protein is 30% and 47.7%.

The crown and tip starch together compares exactly with endosperm 3 and amounts to 34% of the kernel, compared with 17.6% for the latter. The protein content was 6.7% and the proportion of the total 24%, compared with 5.5% and 11% respectively.

The germ content was a little less, 9.6% compared with 11.7% for embryo and scutellum combined, the protein content being similar in each case.

The present results are in good agreement with those of Earle, Curtis, and Hubbard (3), who examined eleven varieties of corn, the average protein content of which was 10.3%, in somewhat less detail than did Hopkins, *et al.* (10). The bran and tip cap together correspond to the pericarp of the present work and as an average for all the varieties comprised 6.2% of the kernel, compared with 6.5% for the pericarp fraction. They contained about 4.8% of protein and 2.8% of the protein of the kernel, compared with 3% and 2.2% respectively. The endosperm comprised 82% of the kernel and contained 9.4% protein and 74.8% of the total protein compared with 79.5%, 8.1%, and 71% for the sum of endosperm 1, 2, and 3. The germ likewise comprised 11.9% of the kernel and contained 18.8% protein and 22.4% of the total protein, compared with 11.7%, 17.4%, and 22.1% for the combined embryo and scutellum fractions.

In Table I the results of the dissection of maize are compared with those of wheat of similar protein content. The distribution of protein follows the same general pattern in each case, with one or two points of difference. Thus the aleurone layer is much smaller in maize and, having the same protein content, contains a smaller proportion of the protein of the kernel. On the other hand the scutellum is very much larger in maize and contains a larger proportion of the total protein despite its lower protein content.

The pericarp of maize contains less protein but anatomically it would be more correct to compare it with the beeswing of wheat which was 4.3% of the kernel and had a protein content of 1.9%.

In each case 70% of the total protein was found in the endosperm in which exists a decreasing concentration gradient from the outer to the inner regions. This gradient would appear to be steeper in maize than it is in wheat since, though endosperm 2 and 3 have similar protein concentrations in the two cereals, endosperm 1 has twice the concentration in maize that it has in wheat. The latter layer, however, represents a much smaller proportion of the kernel in maize, and it is possible that a similar concentration gradient may be shown in wheat if a smaller proportion were removed.

Such an effect has been shown with respect to niacin (7) and it is very probable that protein is similarly distributed in the endosperm and that the concentration gradient is not markedly different in the two cereals.

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COMMUNICATION TO THE EDITOR

Modification of Sedimentation Test for Soft Wheat and Reconstituted Flours

DEAR SIR:

The baking quality of wheat flour is related to the water-absorption capacity of its gluten proteins. A simple method has been devised by Zeleny[1] as a combined measure of the quantity and degree of gluten swelling. The method worked well for hard wheat flours, but was not wholly satisfactory for soft wheat flours and Micka[2] suggested that it be modified by allowing the suspension to stand for ten or fifteen minutes before reading the volume of sediment.

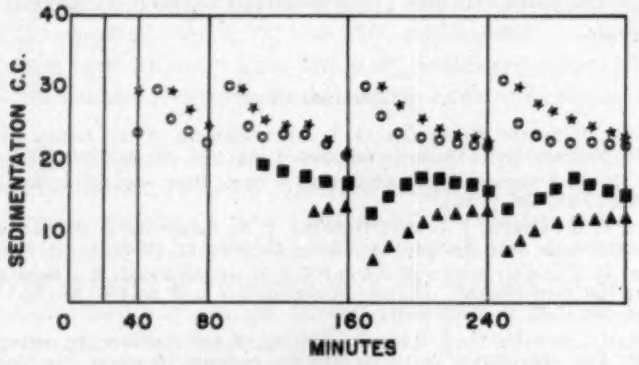

Fig. 1. Sedimentation volumes using four flours: (✱) hard wheat flour; (◎) reconstituted hard wheat flour; (■) soft wheat flour; (▲) reconstituted soft wheat flour. Each vertical line represents remixing. The points shown between each set of vertical lines represent sedimentation readings taken at 10-minute intervals.

An attempt was made to apply the modified method in a comparison of hard wheat, soft wheat, all purpose, and cake flours, and flours reconstituted from fractions of hard and soft wheat flours. A clear reading after 5 to 15 minutes of settling was given only by the hard wheat and all purpose flours. Three poorly defined phases were observed in the other samples—a clear phase at the top shading into a cloudy phase below, and, at the bottom, a solid layer with an indistinct boundary. Rather than settling downwards like the hard wheat flours, the level of sediment in the soft wheat and reconstituted flours as-

[1] Zeleny, Lawrence. A simple sedimentation test for estimating the bread-baking and gluten qualities of wheat flour. Cereal Chem. 24: 465-475 (1947).

[2] Micka, Jan. Zeleny's sedimentation test for biscuit and cracker flours. Trans. Am. Assoc. of Cereal Chemists 10: 188-192 (1952).

cended as accumulation proceeded. Accurate readings were impossible because of the indistinct boundary.

To test whether or not the time of hydration with lactic acid would increase the sharpness of the line of demarkation between the heavy granular sediment at the bottom and the phase directly above it, suspensions of the flours were prepared in the usual manner and allowed to hydrate for longer time intervals. The cylinders were re-inverted at 10, 20, 40, 80, 160, and 240 minutes after the addition of lactic acid. After each of these remixings, readings were recorded at ten minute intervals until the next mixing. These readings are graphed in Fig. 1, each point indicating a clear reading.

With hydration times of 160 minutes and longer, the rate of settling became relatively constant and the upper boundary of the bottom layer was clear after ten minutes standing. Therefore, it was decided to modify the method by allowing the suspensions to hydrate undisturbed for 160 minutes after the addition of lactic acid, then to remix the contents of the cylinders by inverting them 10 times, let them stand for 10 minutes, and read the volume of sediment. The procedure gave clear-cut readings and the volumes of sediment of the hard wheat and soft wheat flours bore the usual relationship to each other and were similar to those reported by Micka[2]. Both hard and soft reconstituted flours gave less sediment than the corresponding original flours.

This modified method was subsequently used in experiments on the quality of biscuits prepared from the reconstituted flours, a report of which will be published at a later date.

Mary C. McAuley

Mary V. Zaehringer

Alice M. Briant

New York State College of Home Economics and New York State Experiment Station at Cornell University, Ithaca, New York

May 14, 1953

BOOK REVIEWS

La Boulangerie Moderne. By R. Calvel. 464 pp. Marc Eyrolles: 61 Boulevard St., Germain, Paris 5. 1952. Price: 1.850 francs.

The author's stated objective is to fill the existing gap in the French technical literature and to provide the student of the baking trade as well as the practicing baker with a fundamental manual of baking technology. Therefore this book is written on an elementary level. The selection of topics is broad and chiefly practical with special attention given to the specific conditions of the French baking industry, composed principally of small size semi-mechanized bakery shops producing a large variety of bakery goods from less uniform raw materials than their American counterpart.

The volume is organized into six parts, each containing several chapters.

In the first portion salient information pertaining to the raw materials used in the baking industry is given. Starting with botanical characteristics of grain, the milling process is described in some detail, and chemical assays and test-baking methods of wheat and flour are discussed. Other ingredients such as yeast, dough improvers, sugars, malt, milk, fats, eggs, and chocolate are discussed briefly. This reviewer regrets the lack of attempt to elucidate the basic roles of the individual ingredients in the baking processes.

The second part deals with dough handling through the make-up stage. Included are discussions of mechanical and hand mixing, fermentation with commercial baker's yeast and with natural starters, and various make-up methods. There is also a description of several types of production equipment.

The third part is devoted to bread baking. The positive contribution of this section is the description of baking ovens from the simplest hand-oven to the more modern equipment. Thermic calculations included will certainly be helpful to the production man.

Part four is concerned with bakery specialties, giving production methods and basic formulas for rye and dietary bread, crescents, rolls, buns, muffins, biscuits, etc. The last chapter of this section deals with the production of bread from exceptionally long extraction flours which were used in France at the time of flour shortage during World War II.

Organization, layout of the bakery shop, and hygiene of personnel are the subjects of the fifth division which is of special interest to the French trade.

The merchandising problems, both technical (e.g. bread staling) and commercial, are discussed in the last part. A chapter on nutritive value of bread is also included.

Karel Kulp
American Institute of Baking
Chicago 11, Illinois

Stability of Shortenings in Cereal and Baked Products. Edited by Robert A. Larsen, Junius M. McIntire and Martin S. Peterson, 46 pp., Research and Development Associates, Food and Container Institute, Inc., Chicago, Illinois, 1953. Distributed free.

In a foreword, H. R. Kraybill points out that this report is the second of a series entitled "Quartermaster Food and Container Institute Surveys of Progress on Military Subsistence Problems." It is comprised of papers and discussion given at a conference at the Institute on January 16, 1953. The main purpose of the confer-

ence was to review the problem of fat stability in cereal and baked products used by the armed forces. An introduction by Donald K. Tressler, Scientific Director of the Quartermaster Food and Container Institute, summarizes the current thinking of the armed forces with respect to shortenings for military rations.

Past and present work at the Food and Container Institute on the stability of shortening in cereal and baked products is reviewed briefly by R. A. Larsen, Chief of the Cereal and Baked Products Division, and J. M. McIntire, Chief of the Dairy, Oil, and Fat Products Division of the Institute.

A specific objective of the conference was to give particular attention to problems involved in the adaptation of animal fats and soybean oil to shortenings for use in miiltary rations. A paper by T. H. McGuine, W. G. Braun, and H. T. Spannuth, reviews methods whereby animal fats can be made into suitable shortenings for various purposes, and a paper by C. E. Morris gives particular attention to interesterification as a means of modifying animal fats to make them more suitable for baked products.

A. E. Bailey reviews general processing methods for the conversion of soybean oil to shortening, and compares the qualities of shortenings prepared from soybean and cottonseed oils. Particular emphasis is given to processing in relation to the reversion problem. J. C. Cowan deals primarily with the fundamental causes of flavor reversion and discusses the role of such factors as fatty acid composition, peroxides, and metal inactivators.

The industrial uses of fats in food products are discussed in the final three papers. Their authors and titles are: Jan Micka, "The Use of Animal Fats in Crackers and Cookies," T. Hollingshead, "The Use of Vegetable Fats in Crackers and Cookies," and W. H. Goss, "The Use of Shortenings in Prepared Mixes."

Although the main emphasis of the conference was on stability problems in military rations, the scope of this collection of papers is somewhat broader; some of them give considerable attention to overall processing methods whereby cheaper fats, notably lard and soybean oil, can be adapted to various shortening purposes. Also, most of the papers review information that is important to the use of shortenings in cereal and baked products for civilian use as well as in military rations.

W. O. Lundberg
The Hormel Institute
Austin, Minnesota, and
Department of Agricultural Biochemistry
University of Minnesota
St. Paul, Minnesota

Cereal Chemistry

EDITORIAL POLICY

Cereal Chemistry publishes scientific papers dealing with raw materials, processes, or products of the cereal industries, or with analytical procedures, technological tests, or fundamental research, related thereto. Papers must be based on original investigations, not previously described elsewhere, which make a definite contribution to existing knowledge.

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SUGGESTIONS TO AUTHORS

General. Authors will find the last volume of *Cereal Chemistry* a useful guide to acceptable arrangements and styling of papers. "On Writing Scientific Papers for Cereal Chemistry" (*Trans. Am. Assoc. Cereal Chem.* 6:1–22. 1948) amplifies the following notes.

Authors should submit two copies of the manuscript, typed double spaced with wide margins on 8½ by 11 inch white paper, and all original drawings or photographs for figures. If possible, one set of photographs of figures should also be submitted. Originals can then be held to prevent damage, and the photographs can be sent to reviewers.

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Use names, not formulas, for text references to chemical compounds. Use plural verbs with quantities. (6.9 g. were). Figures are used before unit abbreviations (3 ml.), and % rather than "per cent" is used following figures. All units are abbreviated and followed by periods, except units of time, which are spelled out. Repeat the degree sign (5°–10°C.). Place 0 before the decimal point for correlation coefficients ($r = 0.95$). Use * to mark statistics that exceed the 5% level and ** for those that exceed the 1% level; footnotes explaining this convention are no longer required. Type fractions on one line if possible, e.g., $A/(B + C)$. Use lower case for farinograph, mixogram, etc., unless used with a proper name, i.e., Brabender Farinograph. When in doubt about a point that occurs frequently, consult the Style Manual or the Dictionary.

For more detailed information on manuscript preparation see *Cereal Chem.* **30:** 351–352 (1953).

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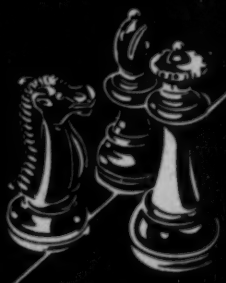

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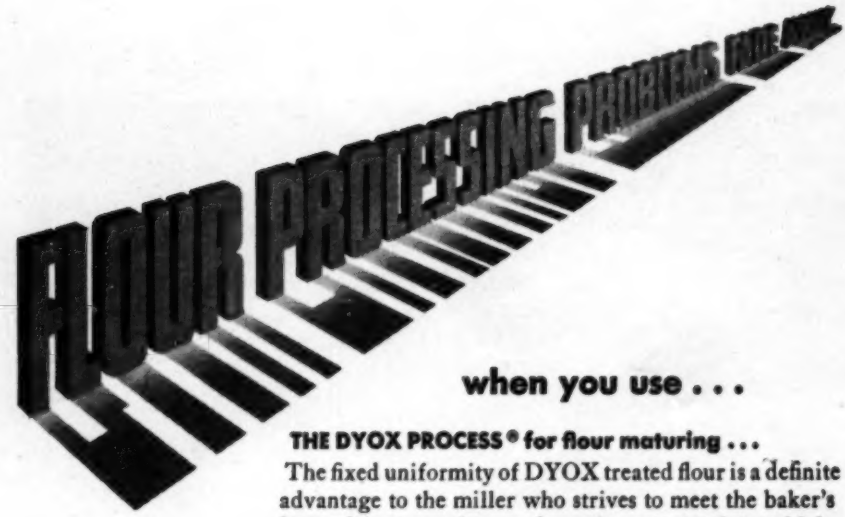

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